Additional file 1

An improved *Escherichia coli* strain to host gene regulatory networks involving both the AraC and LacI inducible transcription factors

Manjunatha Kogenaru[1,2,3*] and Sander J. Tans[1]

[1]FOM Institute for Atomic and Molecular Physics, Science Park 104, 1098 XG Amsterdam, The Netherlands,

[2,3]Present address: [2]EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain. [3]Universitat Pompeu Fabra (UPF), 08003 Barcelona, Spain.

[*]Corresponding author

Email addresses:

MK: mkogenaru@yahoo.com

SJT: tans@amolf.nl

1. Strain engineering

We selected a 40 base pairs (bps) regions from the 5` and 3` ends of the *lacI* as a homology arms and added this to the either ends of the chloramphenicol acetyltransferase (*cat*) selection cassette. This cassette is in turn flanked by the Cre recombinase recognition loxP sites[1]. This whole construct was PCR amplified as a single product and digested with 10 U of *DpnI* for 2 hours at 37 °C. This digested product was further purified by loading on to the agarose gel, before transforming into the parental BW27783 strain by electroporation. Prior to this, the BW27783 was transformed with the phage lambda-derived Red recombinase encoding helper plasmid pKD46[2] (GenBank™ Accession number AY048746). The transformants from the *lacI* deletion were selected on the LB agar plate containing 15 µg/ml Chloramphenicol. The resulting chloramphenicol-resistant clones were screened for the positive recombinants by colony PCR. The confirmed positive clone was further colony-purified once under non-selective condition at 37 °C, and subsequently tested for Ampicillin sensitivity to confirm the loss of the helper plasmid. This clone was further verified for the desired mutation.

V2LacI-del_fr
GTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGAGGAAGCGGAATTCCGGACC

V2LacI-del_rv
TCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTACCGTCGACGGATCCCCGG

lacI

>gi|1657477|gb|U73857.1|ECU73857 Escherichia coli str. K-12 substr. MG1655
GAACAACGGGTGATTGGCTGTCTGAATCTGGTGTATATGGCGAGCGCAATGACCATTGAACAGGCAGCGGAAAAGCATCT
TCCGGCGCTACAACGGGTAGCAAAACAGATCGAAGAAGGGGTTGAATCGCAGGCTATTCTGGTGGCCGGAAGGCGAAGCG
GCATGCATTTACGTTGACACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATT
CAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCG
TGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCC
AACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCC
GTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCG
GCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGAT
GACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCAT
CAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCG
CGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAA
ATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGG
CATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGC
GCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTCAACCACCATC
AAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAA
TCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGG
CCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTA
GC

lacI homology arm sequence
loxP sites
Chloramphenicol acetyltransferase antibiotic resistance gene

LacI-del-test_rv
ACGTAAGAGGTTCCAACTTTCACC

LacI-del-test_fr
CCCGCCCTGCCACTCATCGCAGTAC

LacIdel-test2_fr
CGCAGGCTATTCTGGTGGCC

LacIdel-test2_rv
TCACTGCCCGCTTTCCAGTCG

>MK01 sequence at engineered locus
GAACAACGGGTGATTGGCTGTCTGAATCTGGTGTATATGGCGAGCGCAATGACCATTGAACAGGCAGCGGAAAAGCATCT
TCCGGCGCTACAACGGGTAGCAAAACAGATCGAAGAAGGGGTTGAATCGCAGGCTATTCTGGTGGCCGGAAGGCGAAGCG
GCATGCATTTACGTTGACACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATT
CAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGAGGAAGCGGAATTCCGGACCGGCGCGC
CAAAAAAACCCGCCGAAGCGGGTTTTTTTCCCGGGATAACTTCGTATAATGTATGCTATACGAAGTTATCGATTACGCCC
CGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGA
ACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCATGGTGAAAACGGGGGCGAAGAAGTT
GTCCATATTGGCCACGTTTAAATCAAAACTGGTGAAACTCACCCAGGGATTGGCTGAGACGAAAAACATATTCTCAATAA
ACCCTTTAGGGAAATAGGCCAGGTTTTCACCGTAACACGCCACATCTTGCGAATATATGTGTAGAAACTGCCGGAAATCG
TCGTGGTATTCACTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGGAAAACGGTGTAACAAGGGTGAACACTATCCCA
TATCACCAGCTCACCGTCTTTCATTGCCATACGGAATTCCGGATGAGCATTCATCAGGCGGGCAAGAATGTGAATAAAGG
CCGGATAAAACTTGTGCTTATTTTTCTTTACGGTCTTTAAAAAGGCCGTAATATCCAGCTGAACGGTCTGGTTATAGGTA
CATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGAT
TTTTTTCTCCATTTTAGCTTCCTTAGCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCAT
TATGGTGAAAGTTGGAACCTCTTACGTGCCAATCGATAACTTCGTATAATGTATGCTATACGAAGTTATCCCGGGGATCC
GTCGACGGTAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGA

2. DNA constructs

2.1 Construction of the plasmids used in the competition assay

The gene *lacI* along with the promoter *lacI*^{Q} were amplified from pET-28b+ vector (Novagen) as a template. The *eYFP* gene was amplified from the in-house plasmid pLacImCherry-eYFP (M.K. and

S.J.T, unpublished) as a template. Using these two PCR products as a template, an overlap PCR was performed. The resulting PCR product was cloned onto the vector backbone of pLacImCherry-eYFP with XbaI and SalI restriction enzymes (Figure S2a). Similarly, *eYFP* was replaced with *mCherry* to obtain the construct that constitutively express (Figure S2b).

2.2 Construction of the plasmids used in the simultaneous induction assay

The *araC-*P_{BAD} part was PCR amplified from the in-house plasmid that is similar to the pBAD24 cloning vector (GenBank™ Accession number X81837). The resulting PCR product was cloned into pINV-110[3] plasmid backbone using AatII and XhoI restriction enzymes, to obtain an intermediate plasmid, pAraC. The *mCherry* sequence was cloned into the pAraC using EcoRI and XhoI restriction enzymes (Figure S3a).

The gene *lacI* along with its promoter was cloned into the vector backbone of pLacImCherry-eYFP using HindIII and SalI restriction enzymes (Figure S3b).

Figure S1

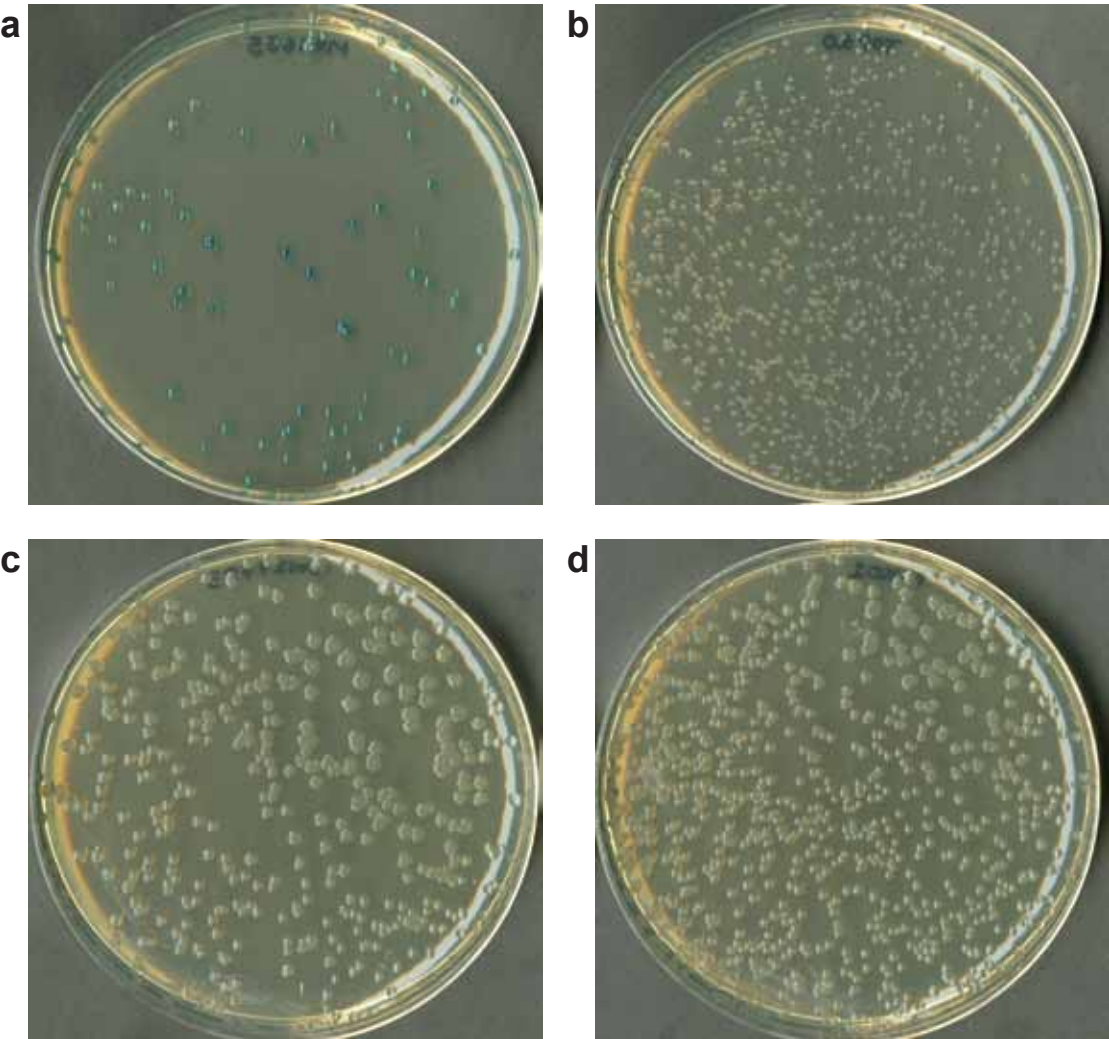
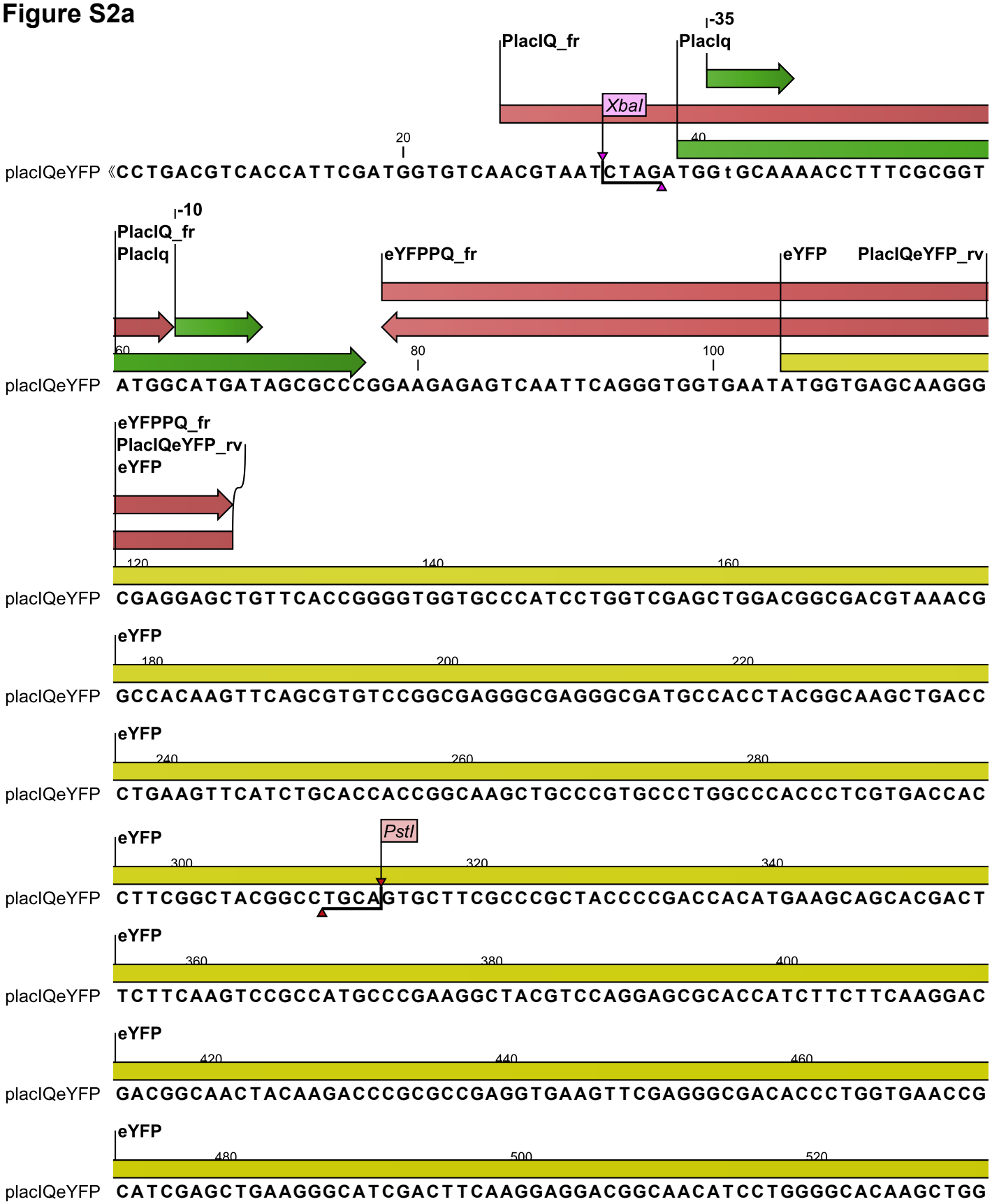


Figure S2a


PlaclQ_fr
-35
Placlq
XbaI
20
40
placlQeYFP 《CCTGACGTCACCATTCGATGGTGTCAACGTAATCTAGATGGtGCAAAACCTTTCGCGGT
-10
PlaclQ_fr
Placlq
eYFPPQ_fr
eYFP
PlaclQeYFP_rv
60
80
100
placlQeYFP ATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATATGGTGAGCAAGGG
eYFPPQ_fr
PlaclQeYFP_rv
eYFP
120
140
160
placlQeYFP CGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACG
eYFP
180
200
220
placlQeYFP GCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACC
eYFP
240
260
280
placlQeYFP CTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCAC
eYFP
PstI
300
320
340
placlQeYFP CTTCGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACT
eYFP
360
380
400
placlQeYFP TCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGAC
eYFP
420
440
460
placlQeYFP GACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCG
eYFP
480
500
520
placlQeYFP CATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGG

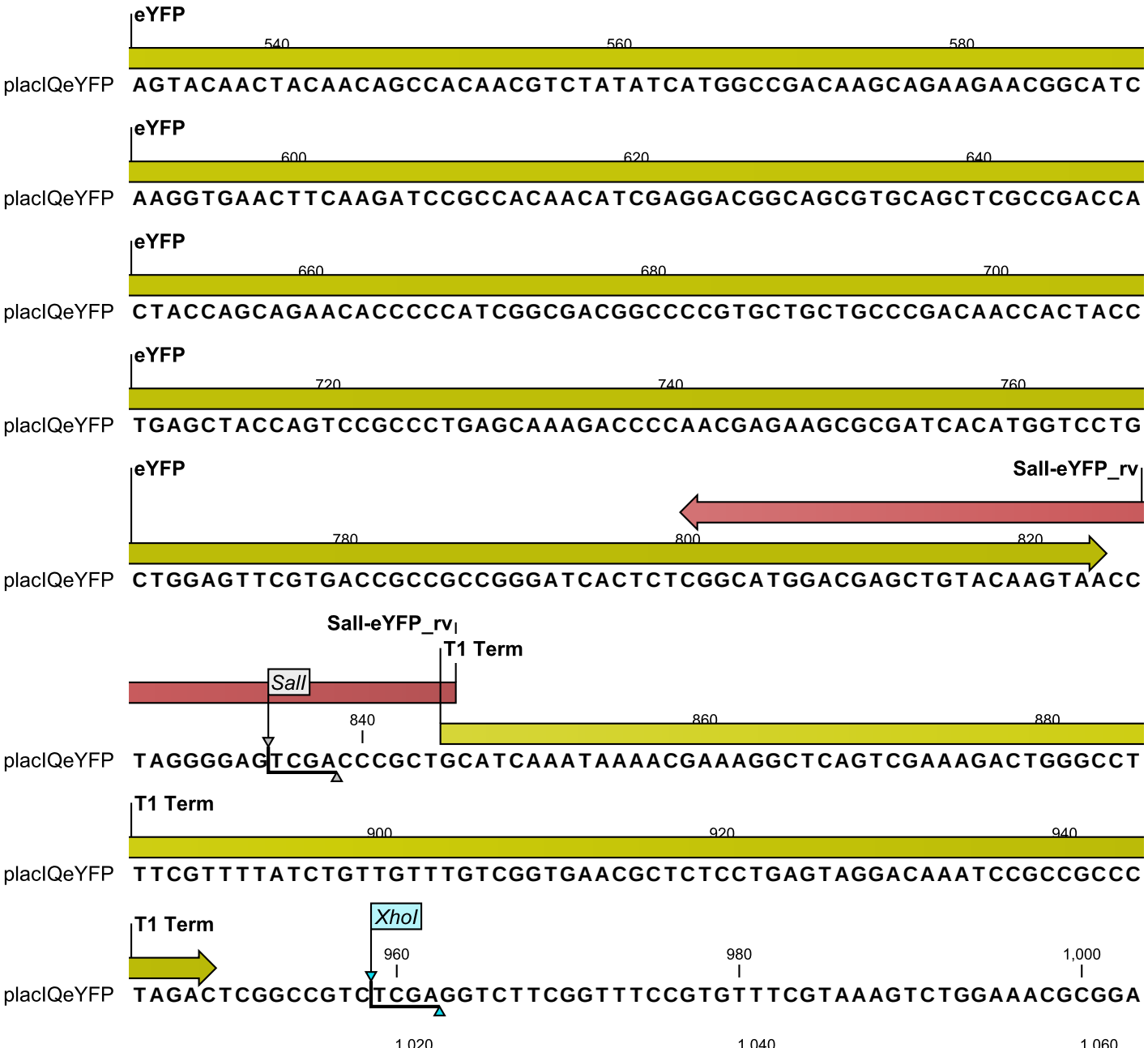
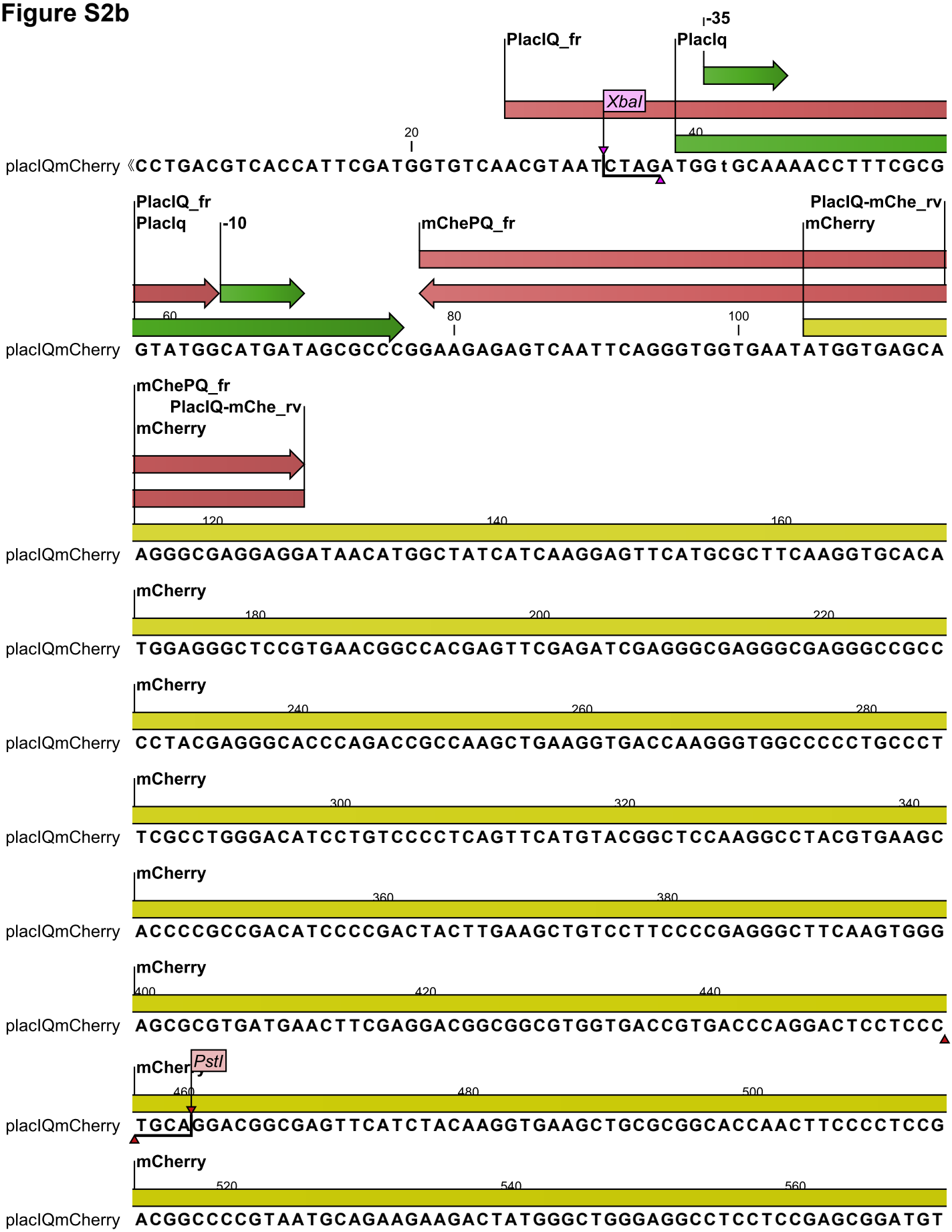

eYFP
540
560
580
placlQeYFP AGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATC
eYFP
600
620
640
placlQeYFP AAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCA
eYFP
660
680
700
placlQeYFP CTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACC
eYFP
720
740
760
placlQeYFP TGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTG
eYFP
SalI-eYFP_rv
780
800
820
placlQeYFP CTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAACC
SalI-eYFP_rv
T1 Term
SalI
840
860
880
placlQeYFP TAGGGGAGTCGACCCGCTGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCT
T1 Term
900
920
940
placlQeYFP TTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCC
T1 Term
XhoI
960
980
1,000
placlQeYFP TAGACTCGGCCGTCTCGAGGTCTTCGGTTTCCGTGTTTCGTAAAGTCTGGAAACGCGGA
1,020
1,040
1,060

Figure S2b


PlacIQ_fr
-35
PlacIq
XbaI
20
40
placlQmCherry 《CCTGACGTCACCATTCGATGGTGTCAACGTAATCTAGATGGtGCAAAACCTTTCGCG
PlacIQ_fr
PlacIq
-10
mChePQ_fr
PlacIQ-mChe_rv
mCherry
60
80
100
placlQmCherry GTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATATGGTGAGCA
mChePQ_fr
PlacIQ-mChe_rv
mCherry
120
140
160
placlQmCherry AGGGCGAGGAGGATAACATGGCTATCATCAAGGAGTTCATGCGCTTCAAGGTGCACA
mCherry
180
200
220
placlQmCherry TGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCC
mCherry
240
260
280
placlQmCherry CCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCT
mCherry
300
320
340
placlQmCherry TCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGC
mCherry
360
380
placlQmCherry ACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGG
mCherry
400
420
440
placlQmCherry AGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCC
mCherry
PstI
460
480
500
placlQmCherry TGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCG
mCherry
520
540
560
placlQmCherry ACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCTCCGAGCGGATGT

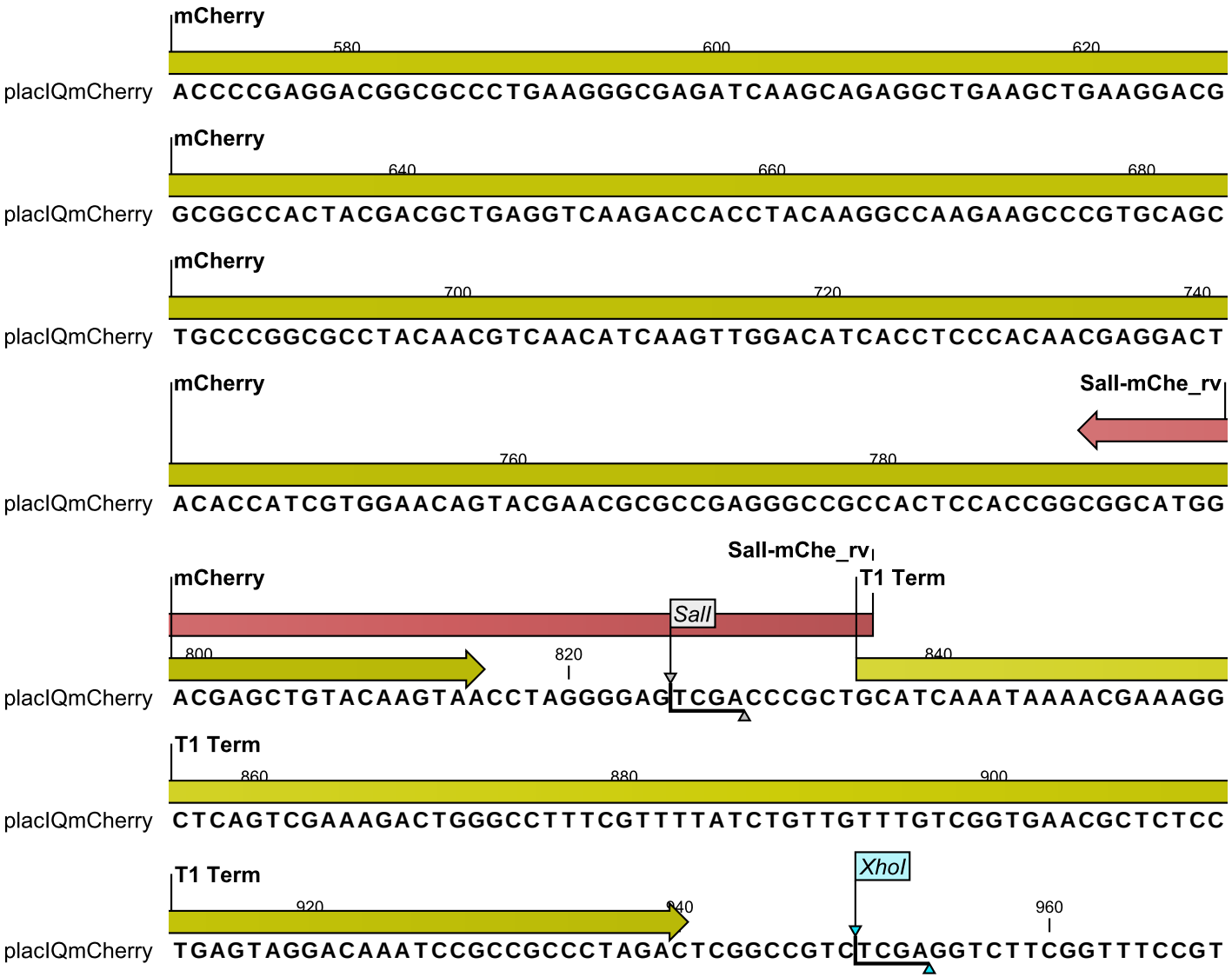
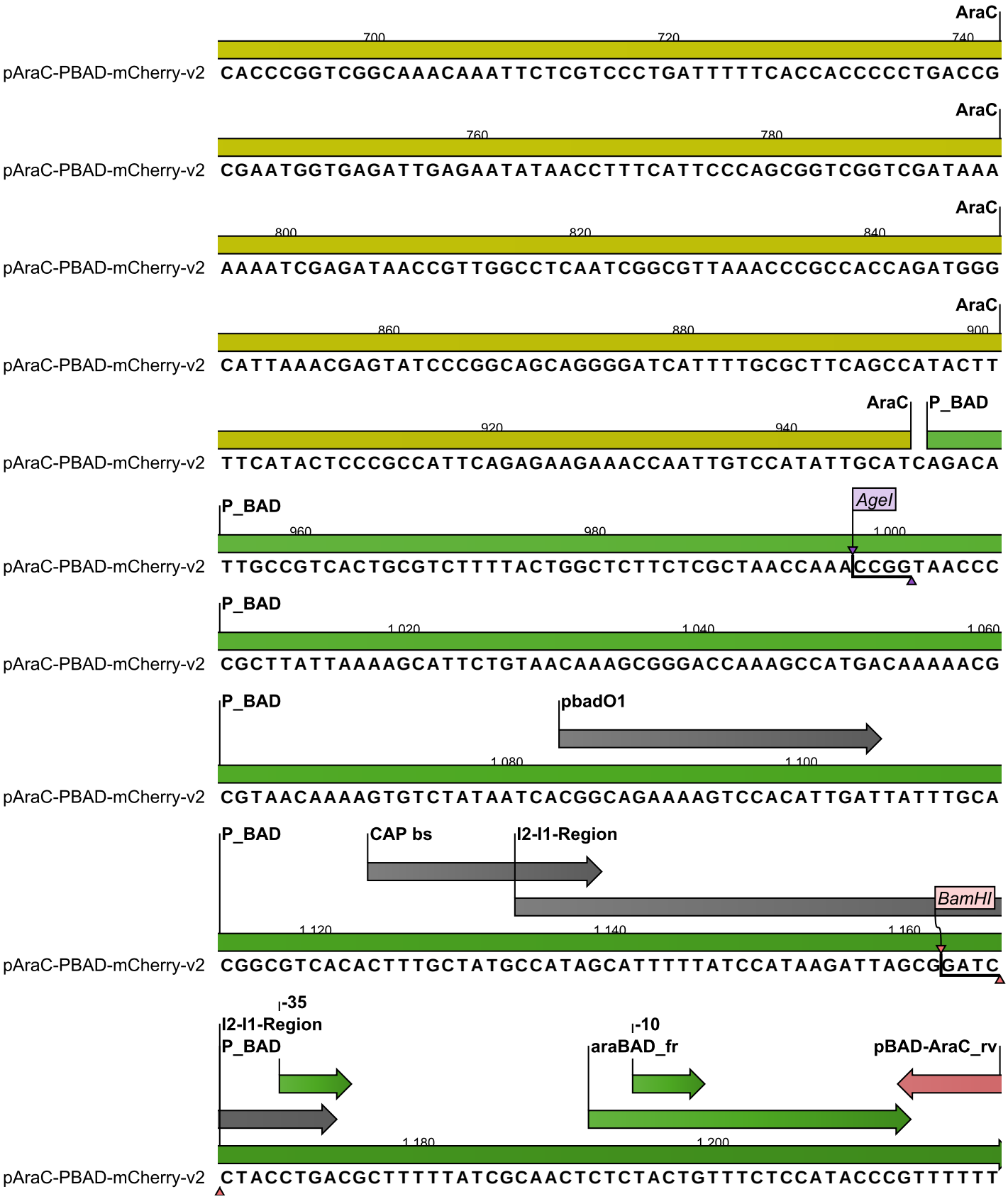
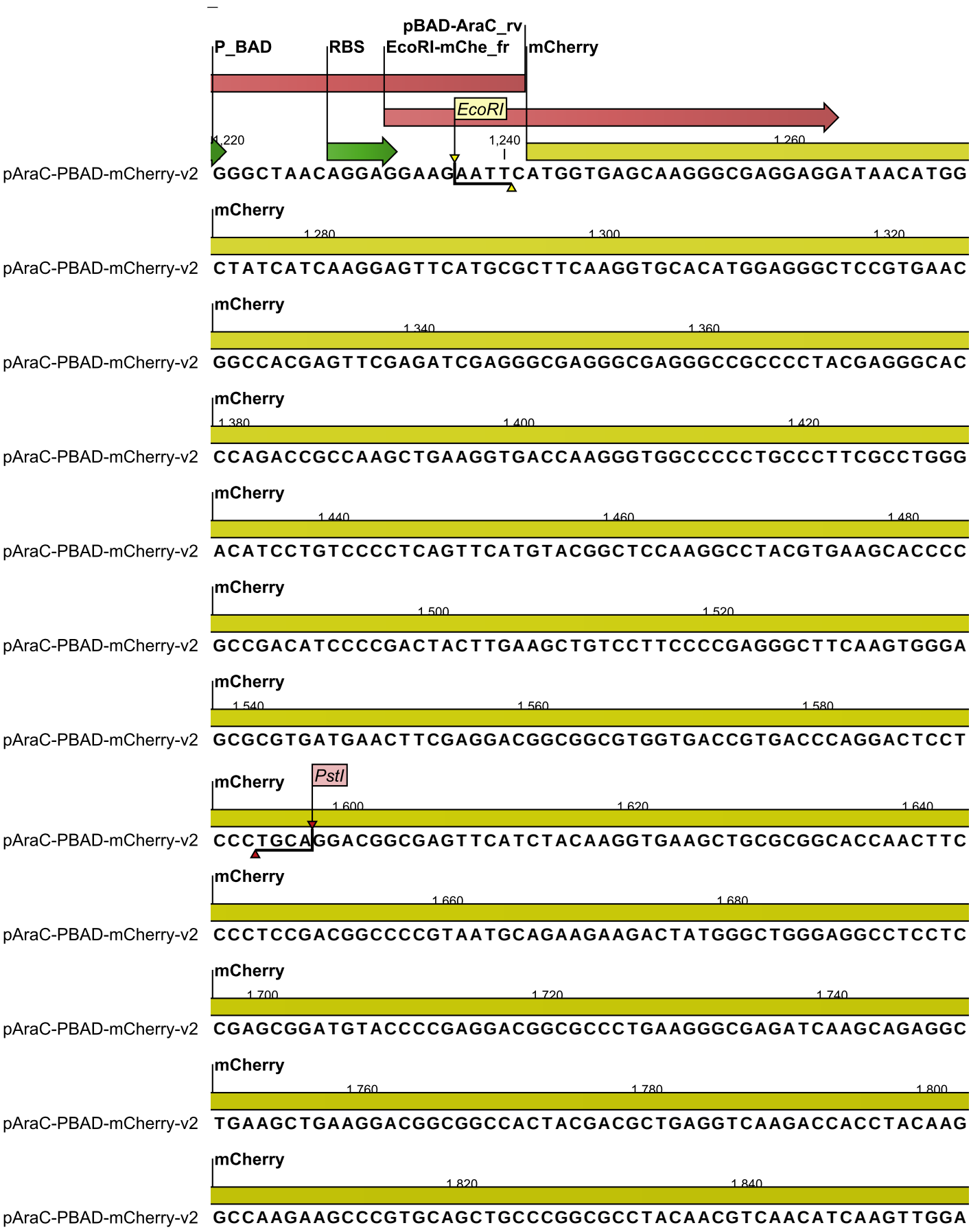

mCherry
580
600
620
placlQmCherry ACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACG
mCherry
640
660
680
placlQmCherry GCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGC
mCherry
700
720
740
placlQmCherry TGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACT
mCherry
SalI-mChe_rv
760
780
placlQmCherry ACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGG
SalI-mChe_rv
mCherry
T1 Term
SalI
800
820
840
placlQmCherry ACGAGCTGTACAAGTAACCTAGGGGAGTCGACCCGCTGCATCAAATAAAACGAAAGG
T1 Term
860
880
900
placlQmCherry CTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCC
T1 Term
XhoI
920
940
960
placlQmCherry TGAGTAGGACAAATCCGCCGCCCTAGACTCGGCCGTCTCGAGGTCTTCGGTTTCCGT

Figure S3a


pBAD-AraC_fr
AraC
20
40
pAraC-PBAD-mCherry-v2 《GACGTCATTGCGTTGCGCTTATGACAACTTGACGGCTACATCATTCACTTTTT
AraC
60
80
100
pAraC-PBAD-mCherry-v2 CTTCACAACCGGCACGGAACTCGCTCGGGCTGGCCCCGGTGCATTTTTTAAAT
AraC
120
140
pAraC-PBAD-mCherry-v2 ACCCGCGAGAAATAGAGTTGATCGTCAAAACCAACATTGCGACCGACGGTGGC
AraC
160
180
200
pAraC-PBAD-mCherry-v2 GATAGGCATCCGGGTGGTGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGT
AraC
220
240
260
pAraC-PBAD-mCherry-v2 CCTCGCGCCAGCTTAAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGAC
EcoRV
AraC
280
300
pAraC-PBAD-mCherry-v2 AGACGCGACGGCGACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATT
AraC
320
340
360
pAraC-PBAD-mCherry-v2 GCTGTCTGCCAGGTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGATTAT
AraC
380
400
420
pAraC-PBAD-mCherry-v2 CCATCGGTGGATGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAAT
AraC
440
460
pAraC-PBAD-mCherry-v2 TGCTCAAGCAGATTTATCGCCAGCAGCTCCGAATAGCGCCCTTCCCCTTGCCC
AraC
480
500
520
pAraC-PBAD-mCherry-v2 GGCGTTAATGATTTGCCCAAACAGGTCGCTGAAATGCGGCTGGTGCGCTTCAT
AraC
540
560
580
pAraC-PBAD-mCherry-v2 CCGGGCGAAAGAACCCCGTATTGGCAAATATTGACGGCCAGTTAAGCCATTCA
AraC
600
620
pAraC-PBAD-mCherry-v2 TGCCAGTAGGCGCGCGGACGAAAGTAAACCCACTGGTGATACCATTCGCGAGC
AraC
640
660
680
pAraC-PBAD-mCherry-v2 CTCCGGATGACGACCGTAGTGATGAATCTCTCCTGGCGGGAACAGCAAAATAT


AraC
700
720
740
pAraC-PBAD-mCherry-v2
CACCCGGTCGGCAAACAAATTCTCGTCCCTGATTTTTCACCACCCCCTGACCG
AraC
760
780
pAraC-PBAD-mCherry-v2
CGAATGGTGAGATTGAGAATATAACCTTTCATTCCCAGCGGTCGGTCGATAAA
AraC
800
820
840
pAraC-PBAD-mCherry-v2
AAAATCGAGATAACCGTTGGCCTCAATCGGCGTTAAACCCGCCACCAGATGGG
AraC
860
880
900
pAraC-PBAD-mCherry-v2
CATTAAACGAGTATCCCGGCAGCAGGGGATCATTTTGCGCTTCAGCCATACTT
AraC
P_BAD
920
940
pAraC-PBAD-mCherry-v2
TTCATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATATTGCATCAGACA
P_BAD
AgeI
960
980
1,000
pAraC-PBAD-mCherry-v2
TTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAAACCGGTAACCC
P_BAD
1,020
1,040
1,060
pAraC-PBAD-mCherry-v2
CGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACG
P_BAD
pbadO1
1,080
1,100
pAraC-PBAD-mCherry-v2
CGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTTGCA
P_BAD
CAP bs
I2-I1-Region
BamHI
1,120
1,140
1,160
pAraC-PBAD-mCherry-v2
CGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCATAAGATTAGCGGATC
-35
I2-I1-Region
P_BAD
-10
araBAD_fr
pBAD-AraC_rv
1,180
1,200
pAraC-PBAD-mCherry-v2
CTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTT


pBAD-AraC_rv
P_BAD
RBS
EcoRI-mChe_fr
mCherry
EcoRI
1,220
1,240
1,260
pAraC-PBAD-mCherry-v2 GGGCTAACAGGAGGAAGAATTCATGGTGAGCAAGGGCGAGGAGGATAACATGG
mCherry
1,280
1,300
1,320
pAraC-PBAD-mCherry-v2 CTATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAAC
mCherry
1,340
1,360
pAraC-PBAD-mCherry-v2 GGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCAC
mCherry
1,380
1,400
1,420
pAraC-PBAD-mCherry-v2 CCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGG
mCherry
1,440
1,460
1,480
pAraC-PBAD-mCherry-v2 ACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCC
mCherry
1,500
1,520
pAraC-PBAD-mCherry-v2 GCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGA
mCherry
1,540
1,560
1,580
pAraC-PBAD-mCherry-v2 GCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCT
mCherry
PstI
1,600
1,620
1,640
pAraC-PBAD-mCherry-v2 CCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTC
mCherry
1,660
1,680
pAraC-PBAD-mCherry-v2 CCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCTC
mCherry
1,700
1,720
1,740
pAraC-PBAD-mCherry-v2 CGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGC
mCherry
1,760
1,780
1,800
pAraC-PBAD-mCherry-v2 TGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAG
mCherry
1,820
1,840
pAraC-PBAD-mCherry-v2 GCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGA

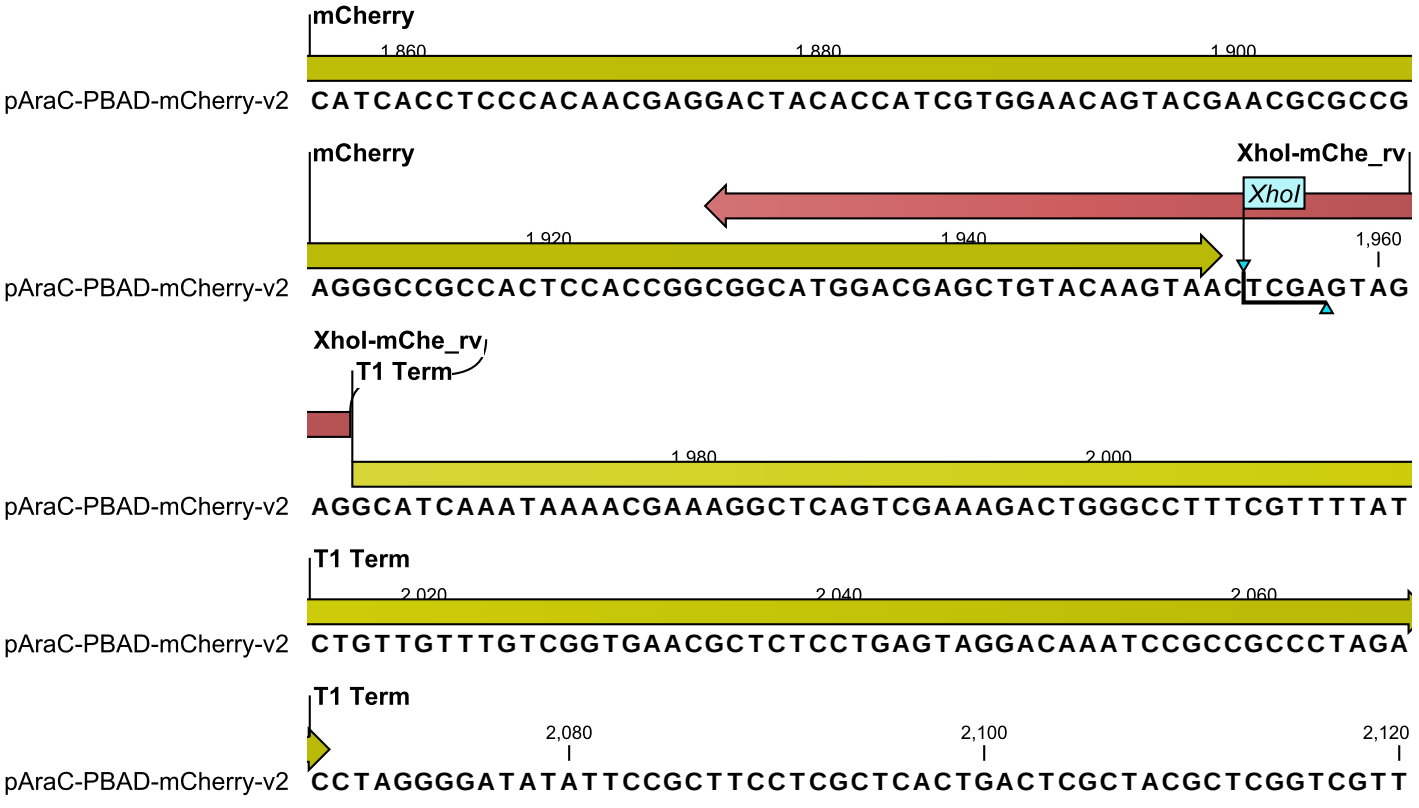
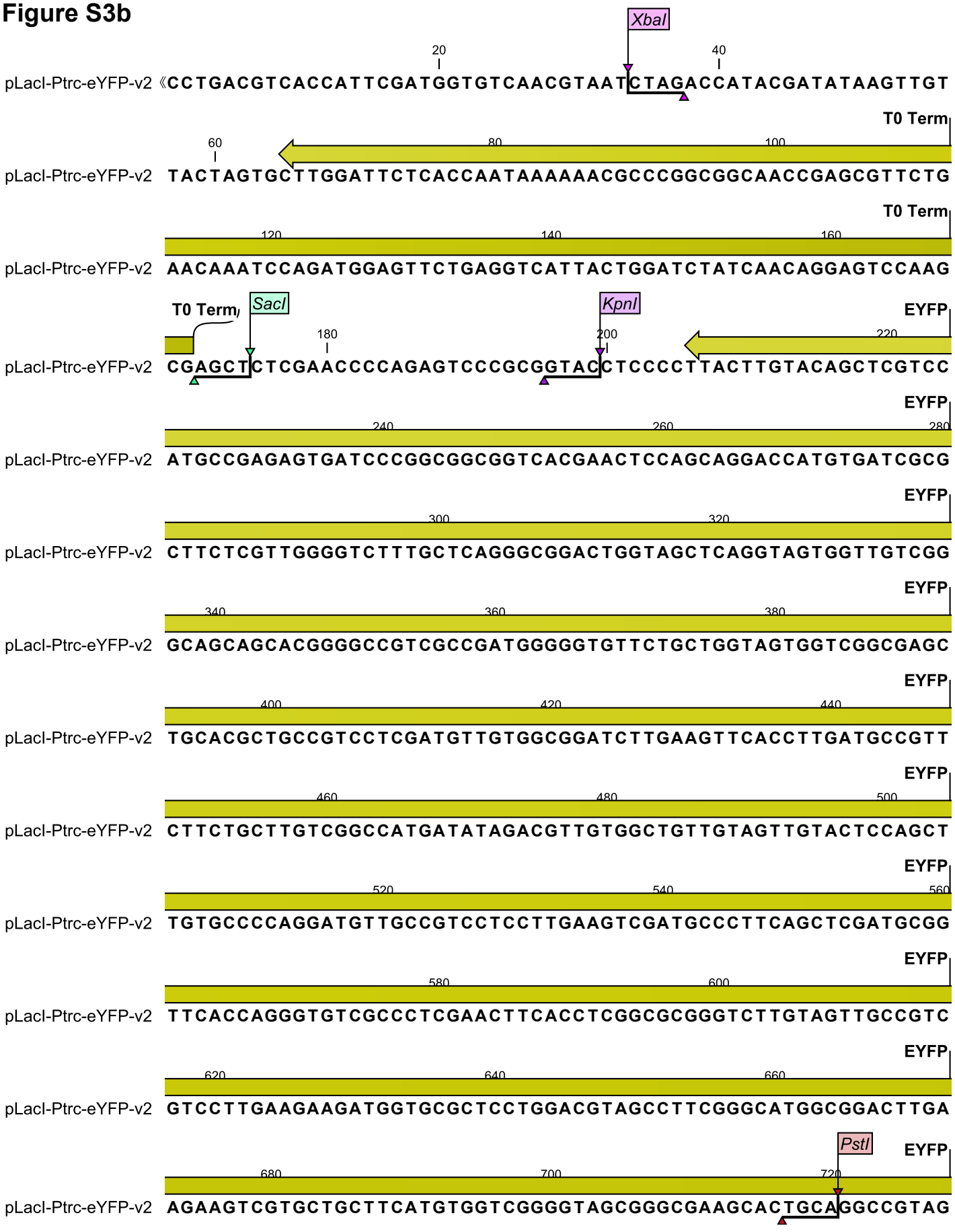
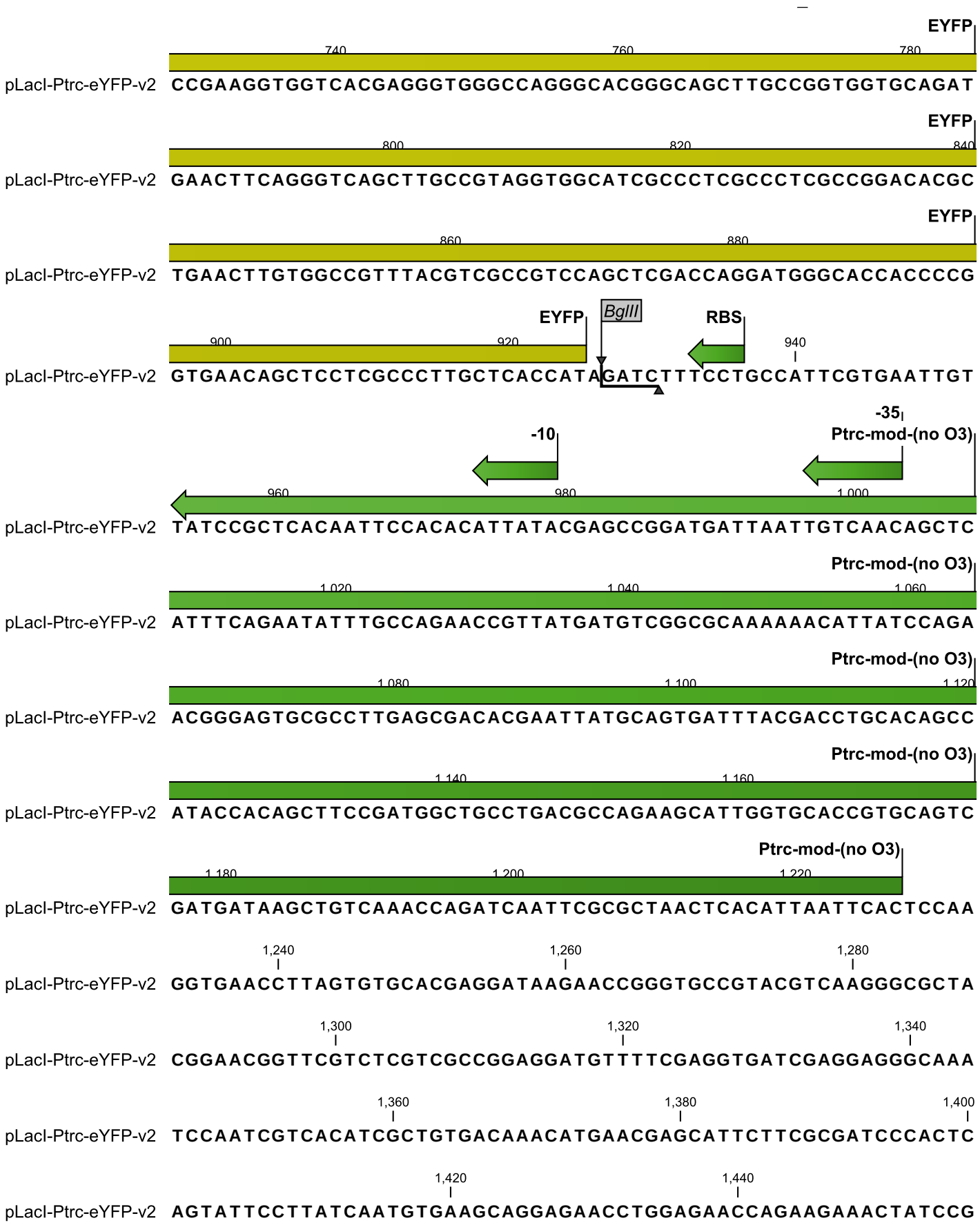
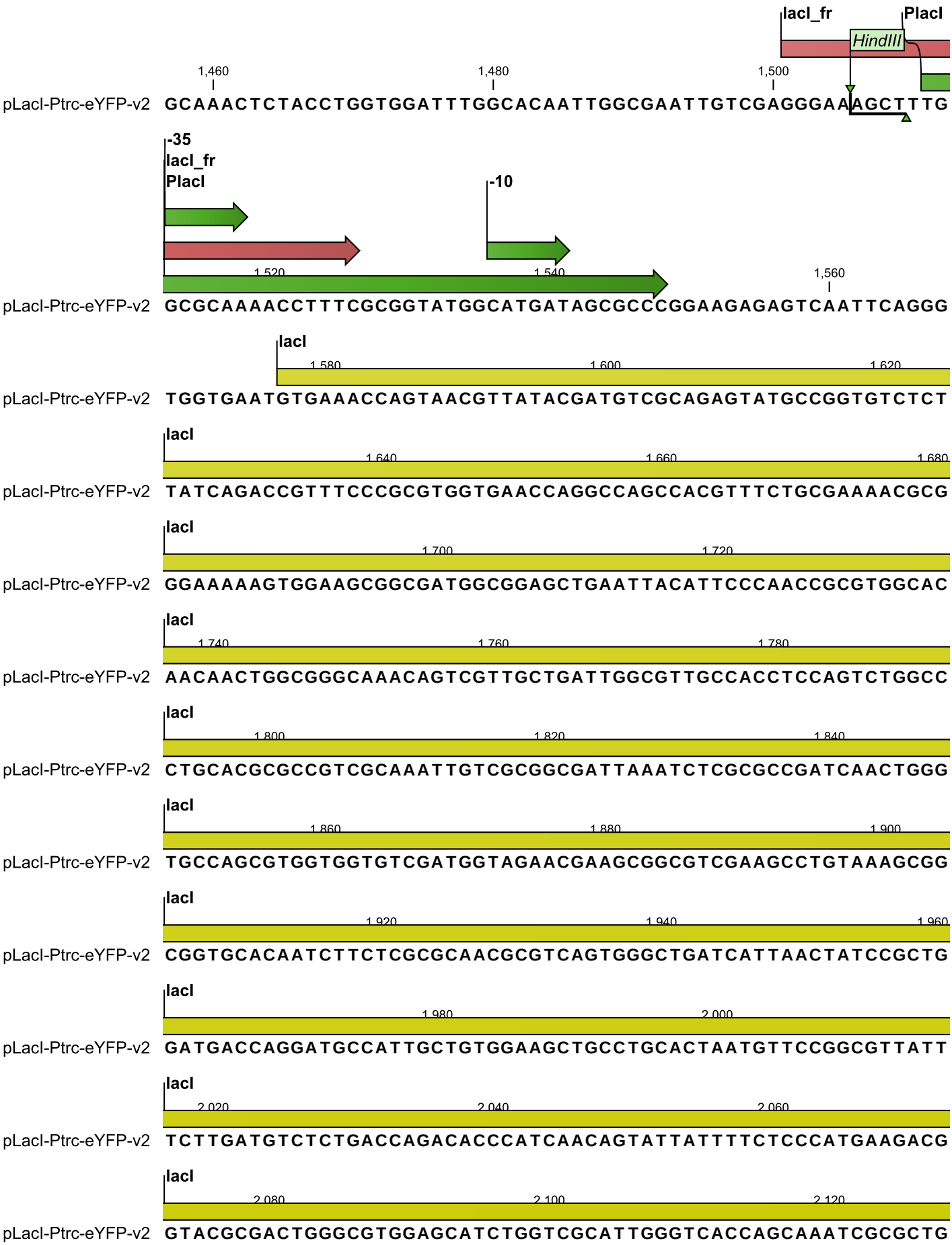
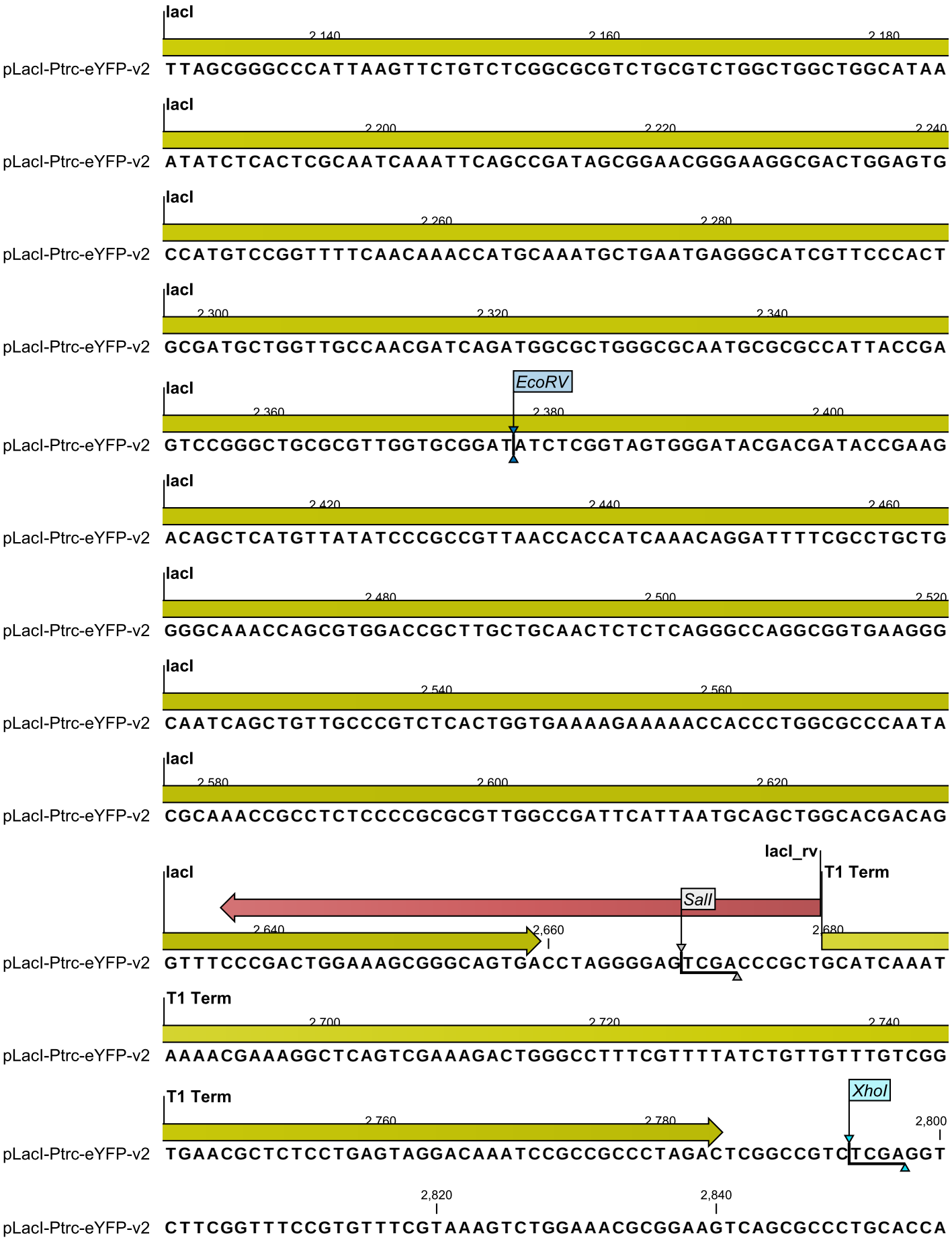

mCherry
1,860
1,880
1,900
pAraC-PBAD-mCherry-v2
CATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCG
mCherry
XhoI-mChe_rv
XhoI
1,920
1,940
1,960
pAraC-PBAD-mCherry-v2
AGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAACTCGAGTAG
XhoI-mChe_rv
T1 Term
1,980
2,000
pAraC-PBAD-mCherry-v2
AGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTAT
T1 Term
2,020
2,040
2,060
pAraC-PBAD-mCherry-v2
CTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGA
T1 Term
2,080
2,100
2,120
pAraC-PBAD-mCherry-v2
CCTAGGGGATATATTCCGCTTCCTCGCTCACTGACTCGCTACGCTCGGTCGTT

Figure S3b


XbaI
20
40
pLacI-Ptrc-eYFP-v2 《CCTGACGTCACCATTCGATGGTGTCAACGTAATCTAGACCATACGATATAAGTTGT
T0 Term
60
80
100
pLacI-Ptrc-eYFP-v2 TACTAGTGCTTGGATTCTCACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTG
T0 Term
120
140
160
pLacI-Ptrc-eYFP-v2 AACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAG
T0 Term
SacI
KpnI
EYFP
180
200
220
pLacI-Ptrc-eYFP-v2 CGAGCTCTCGAACCCCAGAGTCCCGCGGTACCTCCCCTTACTTGTACAGCTCGTCC
EYFP
240
260
280
pLacI-Ptrc-eYFP-v2 ATGCCGAGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATCGCG
EYFP
300
320
pLacI-Ptrc-eYFP-v2 CTTCTCGTTGGGGTCTTTGCTCAGGGCGGACTGGTAGCTCAGGTAGTGGTTGTCGG
EYFP
340
360
380
pLacI-Ptrc-eYFP-v2 GCAGCAGCACGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGC
EYFP
400
420
440
pLacI-Ptrc-eYFP-v2 TGCACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTT
EYFP
460
480
500
pLacI-Ptrc-eYFP-v2 CTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACTCCAGCT
EYFP
520
540
560
pLacI-Ptrc-eYFP-v2 TGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGG
EYFP
580
600
pLacI-Ptrc-eYFP-v2 TTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGCCGTC
EYFP
620
640
660
pLacI-Ptrc-eYFP-v2 GTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGA
PstI
EYFP
680
700
720
pLacI-Ptrc-eYFP-v2 AGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGGCGAAGCACTGCAGGCCGTAG


EYFP
740
760
780
pLacI-Ptrc-eYFP-v2 CCGAAGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGAT
EYFP
800
820
840
pLacI-Ptrc-eYFP-v2 GAACTTCAGGGTCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGC
EYFP
860
880
pLacI-Ptrc-eYFP-v2 TGAACTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCG
EYFP
BglII
RBS
900
920
940
pLacI-Ptrc-eYFP-v2 GTGAACAGCTCCTCGCCCTTGCTCACCATAGATCTTTCCTGCCATTCGTGAATTGT
-35
-10
Ptrc-mod-(no O3)
960
980
1,000
pLacI-Ptrc-eYFP-v2 TATCCGCTCACAATTCCACACATTATACGAGCCGGATGATTAATTGTCAACAGCTC
Ptrc-mod-(no O3)
1,020
1,040
1,060
pLacI-Ptrc-eYFP-v2 ATTTCAGAATATTTGCCAGAACCGTTATGATGTCGGCGCAAAAAACATTATCCAGA
Ptrc-mod-(no O3)
1,080
1,100
1,120
pLacI-Ptrc-eYFP-v2 ACGGGAGTGCGCCTTGAGCGACACGAATTATGCAGTGATTTACGACCTGCACAGCC
Ptrc-mod-(no O3)
1,140
1,160
pLacI-Ptrc-eYFP-v2 ATACCACAGCTTCCGATGGCTGCCTGACGCCAGAAGCATTGGTGCACCGTGCAGTC
Ptrc-mod-(no O3)
1,180
1,200
1,220
pLacI-Ptrc-eYFP-v2 GATGATAAGCTGTCAAACCAGATCAATTCGCGCTAACTCACATTAATTCACTCCAA
1,240
1,260
1,280
pLacI-Ptrc-eYFP-v2 GGTGAACCTTAGTGTGCACGAGGATAAGAACCGGGTGCCGTACGTCAAGGGCGCTA
1,300
1,320
1,340
pLacI-Ptrc-eYFP-v2 CGGAACGGTTCGTCTCGTCGCCGGAGGATGTTTTCGAGGTGATCGAGGAGGGCAAA
1,360
1,380
1,400
pLacI-Ptrc-eYFP-v2 TCCAATCGTCACATCGCTGTGACAAACATGAACGAGCATTCTTCGCGATCCCACTC
1,420
1,440
pLacI-Ptrc-eYFP-v2 AGTATTCCTTATCAATGTGAAGCAGGAGAACCTGGAGAACCAGAAGAAACTATCCG


lacI_fr
PlacI
HindIII
1,460
1,480
1,500
pLacI-Ptrc-eYFP-v2 GCAAACTCTACCTGGTGGATTTGGCACAATTGGCGAATTGTCGAGGGAAAAGCTTTG
-35
lacI_fr
PlacI
-10
1,520
1,540
1,560
pLacI-Ptrc-eYFP-v2 GCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGG
lacI
1,580
1,600
1,620
pLacI-Ptrc-eYFP-v2 TGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCT
lacI
1,640
1,660
1,680
pLacI-Ptrc-eYFP-v2 TATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCG
lacI
1,700
1,720
pLacI-Ptrc-eYFP-v2 GGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCAC
lacI
1,740
1,760
1,780
pLacI-Ptrc-eYFP-v2 AACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCC
lacI
1,800
1,820
1,840
pLacI-Ptrc-eYFP-v2 CTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGG
lacI
1,860
1,880
1,900
pLacI-Ptrc-eYFP-v2 TGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGG
lacI
1,920
1,940
1,960
pLacI-Ptrc-eYFP-v2 CGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTG
lacI
1,980
2,000
pLacI-Ptrc-eYFP-v2 GATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATT
lacI
2,020
2,040
2,060
pLacI-Ptrc-eYFP-v2 TCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACG
lacI
2,080
2,100
2,120
pLacI-Ptrc-eYFP-v2 GTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTG


lacI
2,140
2,160
2,180
pLacI-Ptrc-eYFP-v2
TTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAA
lacI
2,200
2,220
2,240
pLacI-Ptrc-eYFP-v2
ATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTG
lacI
2,260
2,280
pLacI-Ptrc-eYFP-v2
CCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACT
lacI
2,300
2,320
2,340
pLacI-Ptrc-eYFP-v2
GCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGA
lacI
EcoRV
2,360
2,380
2,400
pLacI-Ptrc-eYFP-v2
GTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAG
lacI
2,420
2,440
2,460
pLacI-Ptrc-eYFP-v2
ACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTG
lacI
2,480
2,500
2,520
pLacI-Ptrc-eYFP-v2
GGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGG
lacI
2,540
2,560
pLacI-Ptrc-eYFP-v2
CAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATA
lacI
2,580
2,600
2,620
pLacI-Ptrc-eYFP-v2
CGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAG
lacI_rv
T1 Term
lacI
SalI
2,640
2,660
2,680
pLacI-Ptrc-eYFP-v2
GTTTCCCGACTGGAAAGCGGGCAGTGACCTAGGGGAGTCGACCCGCTGCATCAAAT
T1 Term
2,700
2,720
2,740
pLacI-Ptrc-eYFP-v2
AAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGG
T1 Term
XhoI
2,760
2,780
2,800
pLacI-Ptrc-eYFP-v2
TGAACGCTCTCCTGAGTAGGACAAATCCGCCGCCCTAGACTCGGCCGTCTCGAGGT
2,820
2,840
pLacI-Ptrc-eYFP-v2
CTTCGGTTTCCGTGTTTCGTAAAGTCTGGAAACGCGGAAGTCAGCGCCCTGCACCA

References

1. Watt RM, Wang J, Leong M, Kung HF, Cheah KS, Liu D, Danchin A, Huang JD: **Visualizing the proteome of Escherichia coli: an efficient and versatile method for labeling chromosomal coding DNA sequences (CDSs) with fluorescent protein genes.** *Nucleic Acids Res* 2007, **35:**e37.
2. Datsenko KA, Wanner BL: **One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products.** *PNAS* 2000, **97:**6640-6645.
3. Yokobayashi Y, Weiss R, Arnold FH: **Directed evolution of a genetic circuit.** *PNAS* 2002, **99:**16587-16591.