

**Engineering a terephthalate-responsive PobR biosensor for discovering
PET-degrading microbes via cell-to-cell communication**

**Jung-Ung An^{1,2†}, Seok Jin Oh^{1,3†}, Bong Hyun Sung^{1,3,4,5}, Seung-Goo Lee^{3,4,5}, Kil Koang
Kwon^{1,3,4*}, and Dae-Hee Lee^{1,3,4,5,6*}**

¹Synthetic Biology Research Center and Korea Biofoundry, Korea Research Institute of
Bioscience and Biotechnology (KRIBB), Daejeon 34141, Republic of Korea

²Fermentation Convergence Research Group, Korea Food Research Institute (KFRI), Wanju
55365, Republic of Korea

³Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, University of
Science and Technology (UST), Daejeon 34113, Republic of Korea

⁴Korea Biofoundry, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon
34141, Republic of Korea

⁵Graduate School of Engineering Biology, Korea Advanced Institute of Science and Technology
(KAIST), Daejeon 34141, Republic of Korea

⁶Department of Integrative Biotechnology, College of Biotechnology and Bioengineering,
Sungkyunkwan University, Suwon 16419, Republic of Korea

[†]These authors contributed equally to this work.

*Correspondence: kkkwon@kribb.re.kr; dhlee@kribb.re.kr

This file includes:

Table S1. Primers used in this study.

Table S2. Primers used in mutant library construction.

Table S3. Nucleotide and amino acid sequences of 20 randomly selected clones from the PobR mutant library.

Figure S1. Amino acid sequence alignment of PobR-WT and 20 randomly selected clones from the PobR mutant library.

Figure S2. Dose-response analysis of PobR_2 toward 4-HB and TPA over the 0-0.5 mM range.

Figure S3. Flow-cytometry analysis of 1,000 single clones recovered after FACS enrichment under 1 mM TPA induction.

Figure S4. Secondary screening of recovered PobR variants in the absence and presence of 1 mM TPA.

Figure S5. Proposed important residues of PobR variant structure.

Figure S6. Sequence logo analysis of amino acid distributions at mutagenized PobR positions recovered during TPA-responsive evolution.

Figure S7. Dose-response comparison of PobR_2 and PobR_3 toward 4-HB and TPA over the 0-1.0 mM range.

Figure S8. Growth curves and fluorescence outputs of PobR_4 in different media.

Figure S9. Growth curves and fluorescence outputs of PobR_4 in 1/10-diluted LB medium.

Figure S10. Colony formation and fluorescence of PobR_4 on solid LB agar containing increasing TPA concentrations.

Figure S11. Colony PCR analysis of colonies recovered from the proof-of-concept screening assay.

Figure S12. Structural comparison of the ligand-binding pockets of wild-type PobR and PobR_v10 with docked TPA.

45 **Table S1. Primers used in this study.**

Primer	Sequence (5'-3')
Vector1_F	GAATTCAATGGCGATGACGCATCCTCACGATAAT
Vector1_R	ATGAGCAAAGGTGAAGAACTGTTACCGGCGTTG
PobR_F	TCATCGCCATTGAATTCTTATACCAAATTACGCA
PobR_R	TCTTCACCTTTGCTCATAACATCATCCTTGCTAT
Vector2_F	AGGACTGAGCTAGCCGTAAAGCGGCCGCGAATTCTTATAC
Vector2_R	TGTTATCCGCTTTAATTAAAAATGGCGATGACGCATCCTC
mMucK_F	GAGGATGCGTCATCGCCATTTTTAATTAAAGCGGATAACA
mMucK_R	GTATAAGAATTGCGGGCCGCTTTACGGCTAGCTCAGTCCT
Vector3_F	AAATTAAAGAGGAGAGAAAATGAGCAAAGGTGAAGA
Vector3_R	CATATGTATATCTCCTTAACATCATCCTTGCTAT
RacE_F	ATAGCAAGGATGATGTTAAGGAGATATACATATG
RacE_R	TCTTCACCTTTGCTCATTTTCTCCTCTTTAATTT

46

47

48 **Table S2. Primers used in mutant library construction.**

Primer	Sequence (5'-3')
L141del_F	TTACCCCAGCAAGATAATCGCTATAGCCCCTATGGG
L141del_R	CCCATAGGGGCTATAGCGATTATCTTGCTGGGGTAA
S118_NNK_F	ACCAGCCTCACCTTTNNK*ATTGTGGTGCTCGAT
S118_NNK_R	ATCGAGCACCACAATMNNAAGGTGAGGCTGGT
V143_NNK_F	CAAGATAATTTGCGCNNKAGCCCCTATGGGGCG
V143_NNK_R	CGCCCCATAGGGGCTMNNGCGCAAATTATCTTG
M148_NNK_F	TATAGCCCCTATGGGNKCATTTGGGTAATCGT
M148_NNK_R	ACGATTACCCAAATGMNNCCCATAGGGGCTATA
T159_NNK_F	TTGCCTGCCCACGCCNNKACTACCGGTAAAGTA
T159_NNK_R	TACTTTACCGGTAGTMNNGGCGTGGGCAGGCAA
S160_NNK_F	CCTGCCCACGCCATCNNKACCGGTAAAGTATTA
S160_NNK_R	TAATACTTTACCGGTMNNGATGGCGTGGGCAGG
S212_NNK_F	TCGGACTACTGTTTGNNKACTGAGGAATTTGAA
S212_NNK_R	TTCAAATTCCTCAGTMNNCAAACAGTAGTCCGA
H216_NNK_F	TTGGCTACTGAGGAANNKGAAGTGGGTATGATT
H216_NNK_R	AATCATACCCAGTTCMNNTTCCTCAGTAGCCA
L220_NNK_F	GAATTTGAACTGGGTNNKATTGCGATTGCGGTA
L220_NNK_R	TACCGCAATCGCAATMNNACCCAGTTCAAATTC
M241_NNK_F	GCAGCACTTAATTGTNNKTCACAAACCAATCGG
M241_NNK_R	CCGATTGGTTTGTGAMNNACAATTAAGTGCTGC

* Degenerate bases are indicated according to the IUPAC nucleotide code: N = A/T/G/C, K = G/T, and M = A/C. NNK codons were used for site-saturation mutagenesis of the indicated PobR residues.

Table S3. Nucleotide and amino acid sequences of 20 randomly selected clones from the PobR mutant library.

Name	Nucleotide and amino acid sequences
PobR_Lib_1	CCGAATATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAATTAC GCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCACCCG ATTGTTTGTGACATACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCGCA ATCGCAATCAGACCCAGTTCATGTTCTCAGTAGACAAACAGTAGTCCGATTGCCGAACAGCATCTA ATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCAAT CCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGTGATGGCGTGCGCA GGCAAACGATTACCCAAATGCGCCCCATAGGGGCTCACGCGATTATCTTGCTGGGGTAAATAACTA CGCGCAACAGGTACAATTCATGCTCATCGAGCACCACAATTGAAAAGGTGAGGCTGGTTTGAGC ACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAAA AACGTAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTAAGTG TCTTAAATAACGCCGTGCAGCTGTTCTGCTTATACAGTACGCTCTGCAACCTGTGTACGTTCACT CGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTTC CGTACGTATTTCTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAATC CAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGAT GTCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAGA ACTGTTTACCGGCGTTGTGCCGATTCTGGTGAACTGGAAGGCGATGTGAACGGTCACAAATTCAC CGTGCGTGGTGAAGGGAAAGCGAATGCCCCATTGGCAAACCTGAAACGCTGAAATTTATCTGCAC CACCGGCAAAATGGCGGTGGCGGGGGCGAAACCTGGTGACACCCCGACCCAAGGGGGGTGA CGGGTTTATCCCCC MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKTLK FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFISIVVLDEHEVVPVARSY LPQQDNRVSPYGAHLGNRLPAHAITTKGVLLSVLDREVQIEWIEKYGLKRLTPYITIDEHTFLETDLA VRQSDYCLSTEEHELGLIAIAPVVLNAQGLTIAALNCMSQTNRVQPQYLIDQVLP LLRNTANELRNL V
PobR_Lib_2	CCGGAGGGATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAAT TACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCACC CGATTGGTTTGTGACAGACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCG CAATCGCAATAAAACCCAGTCTTTTTCTCAGTTTTCAAACAGTAGTCCGATTGCCGAACAGCATCT AATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA ATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGGTGGCGTGGG CAGGCAAACGATTACCCAAATGCCTCCCATAGGGGCTAATGCGATTATCTTGCTGGGGTAAATAACT ACGCGCAACAGGTACAATTCATGCTCATCGAGCACCACAATTGCAAAGGTGAGGCTGGTTTGAG CACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAA AAACGTAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTAAGT GTCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACAGTACGCTCTGCAACCTGTGTACGTTCACT

TCGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTT
CCGTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAAT
CCAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGA
TGTCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAG
AACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGGCGATGTGAACGGTCACAAATTC
ACCGTGCGTGGTGAAAGTGAAAGCGATGCCCCGATTGGCAAATGGACGCTGAAAATTTCTGCAC
CACCGGCAAATGGCGGTGGCGGGGGCGAAACCTGGTGACACCCCGACCCAAGGGGTTTAAC
GGGT

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLSLFAIVVLDEHEVVPVARSY
LPQQDNRISPYGRHLGNRLPAHATSTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
VRQSDYCLKTEEKELGFIAIVPVLNAQGLTIAALNCLSQTNRVQPQYLIDQVLP LLRNTANELRLNV**

PobR_Lib_3

CCGAATTATTGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAATTA
CGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCACCC
GATTGGTTTGTGACATACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCGC
AATCGCAATCATACCCAGTTCAAATTCCTCAGTAGCCAAACAGTAGTCCGATTGCCGAACAGCATCT
AATGTTTCTAAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA
ATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGGTGGCGTGGG
CAGGCAAACGATTACCCAAATGCATCCCATAGGGGCTCACGCGATTATCTTGCTGGGGTAAATAAC
TACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTGAAAAGGTGAGGCTGGTTTGAG
CACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAA
AAACGTAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTAAGT
GTCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCACTACGCTCTGCAACCTGTGTACGTTTCA
TCGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTT
CCGTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAAT
CCAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGA
TGTCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAAGGATGATGTTATGAGCAAAGGTGAA
GAACTGTTTACCGGCGTTGTGCCGATTCTGGTGAAATGGGATGGCGATGTGAACGGTCACAAATT
CAGCGTGCGTGGGGAGAGGGTGAAGGGGAAGCCCCCAATGGGCAACATGGACCCTGAAAATTTA
TTTGGCCACCG

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLSLFSIVVLDEHEVVPVARSY
LPQQDNRVSPYGMHLGNRLPAHATSTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLATEEFELGMIAIVPVLNAQGLTIAALNCMSQTNRVQPQYLIDQVLP LLRNTANELRLN
LV**

PobR_Lib_4

ACGGAGGGGATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAA
TTACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCAC
CCGATTGGTTTGTGACGCACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACC

GCAATCGCAATCTTACCCAGTTCATATTCCTCAGTAATCAAACAGTAGTCCGATTGCCGAACAGCAT
CTAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTTC
AATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGCCCAGGCGTGG
GCAGGCAAACGATTACCCAAATGCTGCCCATAGGGGCTCGAGCGATTATCTTGCTGGGGTAAATAA
CTACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTGTAAAGGTGAGGCTGGTTTGA
GCACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGA
AAAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAA
GTGTCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTACAGTTC
AGTCGTTGTCTGTCAATCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATC
TTCCGTACGTATTTCTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAA
TCCAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAG
ATGTCTTTTAAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAA
GAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGATGGCGATGTGAACGGTCACAAATTC
AGCGTGCGTGGGTGAAAGGGAAAGGGAATGCCCCCAAT

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKTLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFTIVVLDEHEVVPVARSYL
PQQDNRSSPYGQHLGNRLPAHAWATGKVLLSVLDREVQIEWIEKYGLKRLTPYITDEHTFLETLD
AVRQSDYCLITEEYELGKIAIVPVLNAQGLTIAALNCASQTNRVQPQYLIDQVPLLRNTANELRNL
V**

PobR_Lib_5 CCGAAGGTATTGCGCCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAA
ATTACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCA
CCCGATTGGTTTGTGACGTACAATTAAGTGTGCAATGGTAAGCCCTGTGCGTTTAAACGGGTAC
CGCAATCGCAATCATACCCAGTTCATATTCCTCAGTCCACAAACAGTAGTCCGATTGCCGAACAGC
ATCTAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTT
TCAATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGGTGGCGTG
GGCAGGCAAACGATTACCCAAATGCATCCCATAGGGGCTAAAGCGATTATCTTGCTGGGGTAAATA
ACTACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTGTAAAGGTGAGGCTGGTTTG
AGCACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAG
AAAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAA
GTGTCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTACAGTTC
AGTCGTTGTCTGTCAATCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATC
TTCCGTACGTATTTCTCACTTGAATGGGGATGTGCAAGGTATTGGGTGATGTTGTTCCATAACATTCA
AATCCAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTAT
AGATGTCTTTTAAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTG
AAGAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGATGGCGATGTGAACGGTCACAATT
CAGCGTGCGTGGTGAAAGTGAAAGCGATGCCACGATTGGCAAAGTACGCTGAAATTATCTGCAC
CCCGGCAAAGTGGCGGTGCCGGGCCAAGCTGGTGACCACTGACCTATGGCGTTCAGGGTTTAG
TCCCTATCGGATCCATGAAACGTCCAATTCTTAATCTGCATGCCGAAGGTATGGGCGGAACAAGAA
TATCTTTAAAAATAAGGCCAAAAAAACCGCCCCCTTGAATTTTAAAGGAAACCGGGGACCCCTTTATA

GAGGGGGTTTTTAAA

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFTIVVLDEHEVVPVARSYL
PQQDNRFSPYGMHLGNRLPAHATSTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLDA
VRQSDYCLWTEEYELGMIAIAVPVLNAQGLTIAALNCTSQTNRVQPQYLIDQVLPLLRNTANELRNL
V**

PobR_Lib_6 CCGGAGGGATGCGCTACCCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAA
TTACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAGGTTGATCGATAAGATACTGTGGCTGCAC
CCGATTGGTTTGTGACAGACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACC
GCAATCGCAATCATACCCAGTTCAAATTCCTCAGTAGCCAAACAGTAGTCCGATTGCCGAACAGCA
TCTAATGTTTCTAAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTT
CAATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGTGATGGCGTGG
GCAGGCAAACGATTACCCAAATGCGCCCCATAGGGGCTAAAGCGATTATCTTGCTGGGGTAAATAA
CTACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTGTAAAGGTGAGGCTGGTTTGA
GCACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGA
AAAACGTAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAA
GTGCTCTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTACGTTT
AGTCGTTGTCTGTCAATCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATC
TTCCGTACGTATTTCTCACTGAATGGGGATGTGCAAGGTATTGGGGGATGTTGTTCCATAACATTCA
AATCCAAAATGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGAATTA
TAGATGTCTTTTAAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGT
GAAGAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGGCCGATGTGAACGGTCACA
AATTCAGCGTGCGTGGGTGGAAGGTGAAAGGCGAATGCCACCAATTGGGCAAACCTGGACCCTG
AAAATTTATCTGGCCCCACCGGGAAAATGGCCGGTGGCCGGGGGCCGAAACCTGGGGGACCAC
CCTGGACCAATGGGGG

**MEQHPQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
LGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFTIVVLDEHEVVPVARSYL
PQQDNRFSPYGAHLGNRLPAHAITGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETDAVR
QSDYCLATEEFELGMIAIAVPVLNAQGLTIAALNCLSQTNRVQPQYLIDQVLPLLRNTANELRNLV**

PobR_Lib_7 CCGGAAGGTGATTGCGCTACCCGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCA
AACTACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAGGTTGATCGATAAGATACTGTGGCTGC
ACCCGATTGGTTTGTGACCAACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTA
CCGCAATCGCAATACAACCCAGTTCATCTTCTCAGTAGGCAAACAGTAGTCCGATTGCCGAACAG
CATCTAATGTTTCTAAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATT
TTTCAATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTAACGGTATGGGAGGCGT
GGGCAGGCAAACGATTACCCAAATGCTGCCATAGGGGCTCCGACGATAATCTTGCTGGGGTAA
TAACTACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTGCAAAGGTGAGGCTGGTT
TGAGCACACAGTAGATTTAAAAACGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACT

AGAAAAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATT
TAAGTGTCTTTAAATATCGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGAGTCACG
TTCAGTCGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTA
ATCTTCCGTACGTATTTCTTCACATGAATGGGGATGTGCAAGGTATTGGTTGATGTTGTTCCATAACATT
CAAATCCAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTTGAGCCTTGAT
TATAGATGTCTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGG
TGAAGAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGATGGCGATGTGAACGGTCACAA
ATTCAGCGTGCGTGGTAAAAGTAAAAGGCAATGCCACGATTGGCAAAGTACGCTGAAATTTTCTG
CACCACCGGCAAACCTTGCCGGGGCCGTGGCCGACGCTGGTGACCCCTGACCCTAGGGGGTT
CAGGGTTTTATCCCCTCCCGGATCACATGAAACGTCCCGATTTCTTAAATCGCATGGCCGGAAG
GGGTTGTGGCGAAACGAACAATAACTTTTAAAGAAAAGGAATAAAAAAACCCCCCGTTGAA
ATTTAGGGGACACCCGCGGGACCCCTTAAGTGAAGGGCCCGTTTTTAAAAAA

**MEQHHQYLAHPHSCEEIREDYIAGLAKGLALLEAFGIDRQLNVTQVAERTGISRTAARRYLKTLK
FLGYLDTDEHYFWLTHRVLRFSYSSYLSSAHLPKVAQSFLNLLCAQTSLTFAIVVLDEHEVVPVARSY
LPQQDYRRSPYQHLGNRLPAHASHTVKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLPTEDELGCIAIAPVPLNAQGLTIAALNCWSQTNRVQPQYLIDQVLP LLRNTANELRS
LV**

PobR_Lib_8 CCAAAGTATTGCGCTACCCGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAA
TTACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCAC
CCGATTGTTTTGTGACATACAATTAAGTGTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACC
GCAATCGCAATCACACCCAGTTCATGTTCTCAGTAGACAAACAGTAGTCCGATTGCCGAACAGCA
TCTAATGTTTCTAAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTT
CAATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGTAGGCGTGG
GCAGGCAAACGATTACCCAAATGCATCCCATAGGGGCTATCGCGATTATCTTGCTGGGGTAAATAA
CTACGCGCAACAGGTACAATTCATGCTCATCAAGCACCACAATTGAAAAGGTGAGGCTGGTTGA
GCACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGA
AAAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAA
GTGTCTTTAAATAGCGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTACGTTT
AGTCGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATC
TTCCGTACGTATTTCTCACTTGAATGGGGATGTGCAAGGATATGGGTGAAGGTTGTTCCATAACATTC
AAATCCAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTTGAGCCTTGATTA
TAGATGTCTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGT
GAAGAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGGCGATGTGAACGGTCACAA
ATTCAGCGTGCGTGGTAAAAGTAAAAGCGATGCCACGATTGGCAAAGTACGCTGAAATTTATCTG
CACCACCGGCAAACCTGCCGTTGCCGTGGCCCAAACCTGGTGACCACCTGACCTATGGGGGT
CAGGGTTTTATTGCTTTCCGGAACACATGAAAAGTCCCAAATTTTTTTATTCTGCAGGGCGGGAGG
GGTTTGGGGGAAGACAAAAATTTTAAAAAAAAGGGCGAATAAAAAAACCCCCCTTTTAA
TTTTAAGGGAAACCCGGGAACCCCTTTAAAAAAGGGGCGGTTTTAAAAAAGAAGGAAAATC
CCGGGGCCAACAGGGAAAACCTTTTATCCCGG

MEQPHPYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQLNVTQVAERTGISRTAARRYLKTLKF
LGYLDTDEHYFWLTHRVLRFSYYLSSAHLPKVAQSFLNLLCAQTSLTFSIVVLDEHEVVPVARSYL
PQQDNRDSPYGMHLGNRLPAHAYSTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETDA
VRQSDYCLSTEEHELGVIAIAPVVLNAQGLTIAALNCMSQTNRVQPQYLIDQVLP LLRNTANELRNL
V

PobR_Lib_9 CCGGAGTTATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAATT
ACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCACC
CGATTGTTTGTGACGAACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCG
CAATCGCAATCACACCCAGTTCAGCTTCCTCAGTAGTCAAACAGTAGCCGATTGCCGAACAGCAT
CTAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTC
AATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTACGGCAGGCGTGG
GCAGGCAAACGATTACCCAAATGCCACCCATAGGGGCTATAGCGATTATCTTGCTGGGGTAAATAA
CTACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATCATAAAGGTGAGGCTGGTTTGA
GCACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGA
AAAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAA
GTGCTTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACAGTACGCTCTGCAACCTGTGTACAGTTC
AGTCGTTGTCTGCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATC
TTCCGTACGTATTTCTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAA
TCCAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAG
ATGTCTTTTAAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAA
GAACTGTTTACCGGCGTTGTGCCGATTCTGGTGAACTGGATGGCGATGTGAACGGTCACAAATTC
ACCGTGCCTGGTTA

MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQLNVTQVAERTGISRTAARRYLKTLK
FLGYLDTDEHYFWLTHRVLRFSYYLSSAHLPKVAQSFLNLLCAQTSLTFMIVVLDEHEVVPVARSY
LPQQDNRYSPYGWHLGNRLPAHACRTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLTTEEELGVIAIAPVVLNAQGLTIAALNCSSQTNRVQPQYLIDQVLP LLRNTANELRNL
V

PobR_Lib_10 CCGGAGTATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAATTA
CGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCACCC
GATTGGTTTGTGACATACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCGC
AATCGCAATCATACCCAGTTCATGTTCTCAGTACCCAAACAGTAGTCCGATTGCCGAACAGCATCT
AATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA
ATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGGAGGCGTGGG
CAGGCAAACGATTACCCAAATGCATCCCATAGGGGCTATTGCGATTATCTTGCTGGGGTAAATAACT
ACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTGCAAAGGTGAGGCTGGTTTGAG
CACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAA
AAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAAGT
GTCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACAGTACGCTCTGCAACCTGTGTACAGTTCAG

TCGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTT
CCGTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGGTGATGTTGTTCCATAACATTCAAA
TCCAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGAATATAG
ATGCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAA
GAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGGCGATGTGAACGGTCACAAATT
CAGCGTGCGTGGTGAAAGTGAAAGCGAAGCCCCAATTGGCAAAGTACGCTGAAATTTCTGCCC
CACCGGCAACTGCCGGTGCCGGGGCCAAACCTGGTGACACCCCGACCC

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFAIVVLDEHEVVPVARSY
LPQQDNRNSPYGMHLGNRLPAHASSTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLGTEEHELGMIAIAPVPLNAQGLTIAALNCMSQTNRVQPQYLIDQVPLLRNTANELR
NLV**

PobR_Lib_11 CCGAGGGGATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATTCTTATACCAAAT
TACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAAAGTGGTATCGATAAGATACTGTGGCTGCACC
CGATTGGTTTGTGACTGACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTAAACGGGTACCG
CAATCGCAATATCACCCAGTTCAAATTCCTCAGTAGGCAAACAGTAGTCCGATTGCCGAACAGCATC
TAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA
ATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTTAATTTGGCGTGGGCA
GGCAAACGATTACCCAAATGCGCCCCATAGGGGCTATAGCGATTATCTTGCTGGGGTAAATAACTA
CGCGCAACAGGTACAACCTCATGCTCATCGAGCACCAATTGGAAAGGTGAGGCTGGTTTGAGC
ACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAAA
AACGTAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTAAGTG
TCTTAAATAACGCCGTGCAGCTGTTCTGCTTATACAGTACGCTCTGCAACCTGTGTACGTTCACT
CGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTTC
CGTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAATC
CAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGAT
GTCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAGA
ACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGTGAACGGTCACAAATTCA
CCGGGCGTGGTTGAAAGGGAAAGGCAATGCCCCAATTGGCAAAGTACGCTGAAATTTATCTGC
ACCACCGGCAAAATGGCCGGGGCCGGGGCCAAACCTGGTGACACCCCGACCCAAGGGGGGT
CCGGGGTTTAATCGCCAC

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFAIVVLDEHEVVPVARSY
LPQQDNRYSPYGAHLGNRLPAHAKLTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLPTEEFELGDIAIAPVPLNAQGLTIAALNCQSQTNRVQPQYLIDQVPLLRNTANELRN
LV**

PobR_Lib_12 CCGGAGGGATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATTCTTATACCAAAT
TACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAAAGTGGTATCGATAAGATACTGTGGCTGCACC

CGATTGGTTTGTGAACAACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCG
CAATCGCAATCAGACCCAGTTCACCTTCCTCAGTAGACAAACAGTAGTCCGATTGCCGAACAGCAT
CTAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTC
AATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTCCAGGTGGCGTGG
GCAGGCAAACGATTACCCAAATGAATCCCATAGGGGCTCACGCGATTATCTTGCTGGGGTAAATAA
CTACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTACAAAGGTGAGGCTGGTTTGA
GCACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGA
AAAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAA
GTGCTTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTCACGTTT
AGTCGTGTCTGTCAATCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATC
TTCCGTACGTATTTCTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAA
TCCAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAG
ATGCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAA
GAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGAATGGCGATGTGAACGGTCACAAATT
CAGCGTGCGTGGTGAAGGTGAAGGCGAAGCCCCGATTGGCAAAGTGGACGCTGAAAATTTATCTG
CACCACCGGCAAAATGGCCGGTGCCGGGGCGCAAACCTGGGGACACCCTGGACCATG

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQLNVTQVAERTGISRTAARRYLKTLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFVIVVLDEHEVVPVARSY
LPQQDNRVSPYGIHLGNRLPAHATWTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLSTEEGELGLIAIVPVLNAQGLTIAALNCCSQTNRVQPQYLIDQVPLLRNTANELRN
LV**

PobR_Lib_13 CCGACTGATGCGCTACCCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCTTATACCAAATT
ACGCAGCTCATTGCGAGTATTGCGTAATAAGGTAAACCTGATCGATAAGATACTGTGGCTGCACC
CGATTGGTTTGTGAAAAACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCG
CAATCGCAATCGTACCCAGTTCAGATTCTCAGTACGCAAACAGTAGTCCGATTGCCGAACAGCAT
CTAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTC
AATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTATTGAGGGCGTGGG
CAGGCAAACGATTACCCAAATGCATCCCATAGGGGCTCATGCGATTATCTTGCTGGGGTAAATAACT
ACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATTGAAAAGGTGAGGCTGGTTTGAGC
ACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAAA
AACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAAGTG
TCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTCACGTTTCACT
CGTTGCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTTC
CGTACGTATTTCTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAATC
CAAGATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGAT
GTCTTTTTAATGAGGCGGTACTTTAGAGATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAGA
ACTGTTTACCGGCATTGTGCCATTCTGGTGGAAGTGGATGGCGATGTGAACGGTCACAAATTCACC
GTGCGTGGTGAAGGTGAAAGCGATGCCCGAATGGCCAAATGACCCTTAAATTTATTGCCCCCGGG
CAAAATGGCCGTGCCGGGGCAAACGCGGGTGACACCCGGGACAATGGGGATCCGGGGTTTATC

GCAC

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFSIVVLDEHEVVPVARSY
LPQQDNRMSPYGMHLGNRLPAHALNTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETL
DAVRQSDYCLRTEESLGTIAIAVPVLNAQGLTIAALNCFSTNRVQPQYLIDQVLP LLRNTANELR
NLV**

PobR_Lib_14 CCGGAGGGATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATTCTTATACCAAAT
TACGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAAACCTTGATCGATAAGATACTGTGGCTGCACC
CGATTGGTTTGTGAATCACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTAAACGGGTACCG
CAATCGCAATATCACCCAGTTCATGTTCTCAGTAGGCAAACAGTAGTCCGATTGCCGAACAGCATC
TAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA
ATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGTAGGCGTGGGC
AGGCAAACGATTACCCAAATGCATCCCATAGGGGCTCTTGCGATTATCTTGCTGGGGTAAATACTA
CGCGCAACAGGTACAACCTTCATGCTCATCGAGCACCACAATTAAGGTGAGGCTGGTTTGAGCA
CACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAAAA
ACGTAAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAAGTG
CTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTCACGTTTCAGTC
GTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTTCC
GTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAATCCA
AAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGATGT
CTTTTAAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAGAAC
TGTTTACCGGCGTTGTGCCGATTCTGGTGAACTGGAATGGCGATGTGAACGGTCACAAATTCACC
GGCCCTGGTGAAAAGGGAAGGGATGCCCCCATTGGCAAACCTGGACGCTGAAAATTTATCTGGAC
CACCGGCAAAATGGCGGTGGCCGGGGGCCAAACCTGTGGA

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFLIVVLDEHEVVPVARSYL
PQQDNRKSPYGMHLGNRLPAHAYSTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLDA
VRQSDYCLPTEEHLDIAIAVPVLNAQGLTIAALNCDSQTNRVQPQYLIDQVLP LLRNTANELRNL
V**

PobR_Lib_15 CCGAGGGATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATTCTTATACCAAATTA
CGCAGCTCATTGCGAGTATTGCGTAATAAAGGTAAAACCTTGATCGATAAGATACTGTGGCTGCACCC
GATTGGTTTGTGACATACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTAAACGGGTACCGC
AATCGCAATCGAACCAGTTCATGTTCTCAGTAGCCAAACAGTAGTCCGATTGCCGAACAGCATC
TAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA
ATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTATGGTTGGCGTGGGC
AGGCAAACGATTACCCAAATGATCCCATAGGGGCTCGTGCGATTATCTTGCTGGGGTAAATACTA
CGCGCAACAGGTACAACCTTCATGCTCATCGAGCACCACAATTTGAAAGGTGAGGCTGGTTTGAGCA
CACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAAAA

ACGTAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAAGTGT
CTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTCACGTTTCAGTC
GTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTTCC
GTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGGTGATGTTGTTCCATAACATTCAAATC
CAAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTTGAGCCTGAATTATAGAT
GTCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAGA
ACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGGCGAAGTGAACGGTCACAAATTCA
CCGGGCGTGGGGGAAAGGGAAAGGCGAAGCCCCGAATGGCAAAGTGGAGCCTGAAAATTTATCT
GGCCACCGGGGAAAATGGCGGTGGCGTGGGCCAAAACCTT

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKTLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFQIVVLDEHEVVPVARSY
LPQQDNRTSPYGNHLGNRLPAHANHTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLATEEHELGSIAIAPVVLNAQGLTIAALNCMSQTNRVQPQYLIDQVLP LLRNTANELRN
LV**

PobR_Lib_16 ACGGAGGATGCGCTACCCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATTCTTATACCAAATT
ACGCAGCTCATTGCGAGTATTGCGTAATAAGGTAAAACCTTGATCGATAAGATACTGTGGCTGCACC
CGATTGGTTTGTGACCTACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTAAACGGGTACCG
CAATCGCAATATGACCCAGTTCATCTTCTCAGTACACAAACAGTAGTCCGATTGCCGAACAGCATC
TAATGTTTCTAAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA
ATCCATTCAATTTGACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTATATTTGGCGTGGGCA
GGCAAACGATTACCCAAATGCGCCCCATAGGGGCTCACGCGATTATCTTGCTGGGGTAAATAACTA
CGCGCAACAGGTACAACCTTCATGCTCATCGAGCACCACAATCCAAAAGGTGAGGCTGGTTTGAGC
ACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAAA
AACGTAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTTAAGTG
TCTTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTCACGTTTCAGT
CGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTTC
CGTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGGTGATGTTGTTCCATAACATTCAAAT
CCAAAATGGTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTGAATTATAG
ATGTCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGGATGATGTTATGAGCAAAGGTGA
AGAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGGCGAAGTGAACGGTCACAAAT
TCACCGGGCGGTGGTGAAAAGGTGAAAGGGAATGCCCCATTGGGCAAAGTGGACGCTGAAAAT
TTATCTGGCCACCGGGAAAATGGCGGTGGCCGGGGGCCAAAACCTGGG

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKTLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFWIVVLDEHEVVPVARSY
LPQQDNRVSPYGAHLGNRLPAHAKYTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLCTEEDELGHIAIAPVVLNAQGLTIAALNCRSQTNRVQPQYLIDQVLP LLRNTANELRN
LV**

PobR_Lib_17 CCGGAAGGATGCGCTACCCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATTCTTATACCAAATT

ACGCAGCTCATTGCGCAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCACC
CGATTGGTTTGTGACGCACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCG
CAATCGCAATACAACCCAGTTCATGTTCTCAGTAGACAAACAGTAGTCCGATTGCCGAACAGCATC
TAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTCA
ATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGAAGGCGTGGG
CAGGCAAACGATTACCCAAATGCATCCCATAGGGGCTCACGCGATTATCTTGCTGGGGTAAATAAC
TACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATATGAAAGGTGAGGCTGGTTTGAG
CACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAA
AAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTAAGT
GTCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACAGTACGCTCTGCAACCTGTGTACAGTTCAG
TCGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTT
CCGTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAAT
CCAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGA
TGCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAG
AACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGATGGCGATGTGAACGGTCACAAATTCA
CCGTGCGTGGTGAAGGGGAAAGGCAATGCCCCGATTGGCAAAGTGGACGCTGAAAATTTATCTG
GCCCACCGGGAAAATG

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTDEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFHIVVLDEHEVVPVARSY
LPQQDNRVSPYGMHLGNRLPAHAFSTGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLSTEEHELGCIAIVPVLNAQGLTIAALNCASQTNRVQPQYLIDQVPLLRNTANELRN
LV**

PobR_Lib_18 CCGGAGGGATGCGCTACCGGATATTATCGTGAGGATGCGTCATCGCCATTGAATCCTTATACCAAAT
TACGCAGCTCATTGCGCAGTATTGCGTAATAAAGGTAAACTTGATCGATAAGATACTGTGGCTGCACC
CGATTGGTTTGTGACATACAATTAAGTGCTGCAATGGTAAGCCCCTGTGCGTTTAAACGGGTACCG
CAATCGCAATCAGACCCAGTTCATGTTCTCAGTAGACAAACAGTAGTCCGATTGCCGAACAGCAT
CTAATGTTTCTAAAAAGTATGTTTCATCGGTAATGGTATAAGGCGTGAGGCGCTTCAGACCATATTTTTC
AATCCATTCAATTTGTACTTCACGATCCAGAACAGAAAGTAATACTTTACCGGTAGAGGTGGCGTGGG
CAGGCAAACGATTACCCAAATGCAACCCATAGGGGCTCACGCGATTATCTTGCTGGGGTAAATAAC
TACGCGCAACAGGTACAACCTCATGCTCATCGAGCACCACAATAAAAAAGGTGAGGCTGGTTTGAG
CACACAGTAGATTTAAAAATGACTGTGCCACTTTGGGTAAATGTGCCGAGCTTAAATAAGAACTAGAA
AAACGTAAAACACGATGTGTAAGCCAAAAATAGTGCTCATCGGTATCTAAATAACCCAAAAATTAAGT
GTCTTTAAATAACGCCGTGCAGCTGTTCTGCTTATACAGTACGCTCTGCAACCTGTGTACAGTTCAG
TCGTTGTCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTT
CCGTACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGTGATGTTGTTCCATAACATTCAAAT
CCAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTTGATTATAGA
TGCTTTTTAATGAGGCGGTACTTTAAAAATAGAAAATAGCAAGGATGATGTTATGAGCAAAGGTGAAG
AACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAGTGGATGGCGATGTGAACGGTCACAAATTC
AGCGTGCGTGGTGAAGGTGAAAGGCGATGCCCCGATTGGGAAAGTGAACGCTGAAAATTTTCTG

TTAAATAACGCCGTGCAGCTGTTCTGCTTATACCAGTACGCTCTGCAACCTGTGTCACGTTCAAGTCG
TTGCTGTCAATTCCAAATGCTTCTAAAAGCGCTAAGCCTTTGGCCAAGCCTGCAATGTAATCTTCCG
TACGTATTTCTTCACTTGAATGGGGATGTGCAAGGTATTGGGTGATGTTGTTCCATAACATTCAAATCC
AAAATGGTTTTGTCCGATCATCGGACAGTTGTAATGCTAATCGGATAATTTGAGCCTGAATTATAGATG
TCTTTTAAATGAGGCGGTACTTTAAAAATAGAAAATAGCAGGGATGATGTTATGAGCAAAGGTGAAGAA
CTGTTTACGGCGTTGTGCCGATTCTGGTGGAAGTGGGATGGGCGAAGTGAACGGTCACAAATTCA
GCGTGCGTGGTTGAAAAGGGAAGGGAATGCCCCGA

**MEQHHQYLAHPHSSEEIRTEDYIAGLAKGLALLEAFGIDRQRLNVTQVAERTGISRTAARRYLKLK
FLGYLDTEHYFWLTHRVLRFSSSYLSSAHLPKVAQSFLNLLCAQTSLTFTIVVLDEHEVVPVARSYL
PQQDNRNSPYGMHLGNRLPAHAQATGKVLLSVLDREVQIEWIEKYGLKRLTPYTITDEHTFLETLD
AVRQSDYCLTTEENELGTIAIAVPVLNAQGLTIAALNCPSQTNRVQPQYLIDQVLP LLRNTANELRNL
V**

	10	20	30	40	50	60	70	80	90	100	110	120	130
WT	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFSIV	VLDEHEVVPV ARSYLP
Lib_1	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFSIV	VLDEHEVVPV ARSYLP
Lib_2	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	AIV VLDEHEVVPV ARSYLP
Lib_3	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFSIV	VLDEHEVVPV ARSYLP
Lib_4	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	AIV VLDEHEVVPV ARSYLP
Lib_5	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	AIV VLDEHEVVPV ARSYLP
Lib_6	MEQHPQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	AIV VLDEHEVVPV ARSYLP
Lib_7	MEQHHQYLAH	PHSCEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	AIV VLDEHEVVPV ARSYLP
Lib_8	MEQPHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFSIV	VLDEHEVVPV ARSYLP
Lib_9	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	MIV VLDEHEVVPV ARSYLP
Lib_10	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	AIV VLDEHEVVPV ARSYLP
Lib_11	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	PIV VLDEHEVVPV ARSYLP
Lib_12	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	VIV VLDEHEVVPV ARSYLP
Lib_13	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFSIV	VLDEHEVVPV ARSYLP
Lib_14	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	LIV VLDEHEVVPV ARSYLP
Lib_15	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	QIV VLDEHEVVPV ARSYLP
Lib_16	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	MIV VLDEHEVVPV ARSYLP
Lib_17	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	HIV VLDEHEVVPV ARSYLP
Lib_18	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	FIV VLDEHEVVPV ARSYLP
Lib_19	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	MIV VLDEHEVVPV ARSYLP
Lib_20	MEQHHQYLAH	PHSSEEIRTE	DYIAGLAKGL	ALLEAFGIDR	QRLNVTQVAE	RTGISRTAAR	RYLKTlkFLG	YLDtDEHYFW	LTHRVLRFSS	SYLSSAHLPK	VAQSFLNLLC	AQTSLTFT	AIV VLDEHEVVPV ARSYLP

	140	150	160	170	180	190	200	210	220	230	240	250	260	270
WT	QQDN LRVSPYGMHL	GNRLPAHATS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LSTEEHELGL	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_1	QQDN LRVSPYGAHL	GNRLPAHAIT	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LSTEEHELGL	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_2	QQDN LRVSPYGRHL	GNRLPAHATS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LKTEEXELGLF	IAIAVPVLNA	QGLTIAALNC	LSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_3	QQDN LRVSPYGMHL	GNRLPAHATS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LATEEFELG M	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_4	QQDN LRVSPYQHL	GNRLPAHAWA	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LITEEYELG K	IAIAVPVLNA	QGLTIAALNC	ASQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_5	QQDN LRVSPYGMHL	GNRLPAHATS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LMTEEYELG M	IAIAVPVLNA	QGLTIAALNC	TSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_6	QQDN LRVSPYGAHL	GNRLPAHAIT	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LATEEFELG M	IAIAVPVLNA	QGLTIAALNC	LSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_7	QQDY LRVSPYQHL	GNRLPAHAS H	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LPTEEEDELG C	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRSL V	
Lib_8	QQDN LRVSPYGMHL	GNRLPAHAYS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LSTEEHELGL V	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_9	QQDN LRVSPYGMHL	GNRLPAHACR	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LTEEEAELG V	IAIAVPVLNA	QGLTIAALNC	SSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_10	QQDN LRVSPYGMHL	GNRLPAHAS S	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LGTEEEHELG M	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_11	QQDN LRVSPYGAHL	GNRLPAHAKL	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LPTEEEFELG D	IAIAVPVLNA	QGLTIAALNC	QSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_12	QQDN LRVSPYIHL	GNRLPAHATM	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LSTEEGELGL	IAIAVPVLNA	QGLTIAALNC	CSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_13	QQDN LRVSPYGMHL	GNRLPAHALN	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LRTEESELG T	IAIAVPVLNA	QGLTIAALNC	FSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_14	QQDN LRVSPYGMHL	GNRLPAHAYS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LPTEEEHELGD	IAIAVPVLNA	QGLTIAALNC	DSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_15	QQDN LRVSPYGNHL	GNRLPAHANH	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LSTEEHELGL	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_16	QQDN LRVSPYGAHL	GNRLPAHAKY	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LCTEEDELGH	IAIAVPVLNA	QGLTIAALNC	RSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_17	QQDN LRVSPYGMHL	GNRLPAHAFS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LSTEEHELGL C	IAIAVPVLNA	QGLTIAALNC	ASQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_18	QQDN LRVSPYGLHL	GNRLPAHATS	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LSTEEHELGL	IAIAVPVLNA	QGLTIAALNC	MSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_19	QQDN LRVSPYKHL	GNRLPAHAPC	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LMTEEFELGR	IAIAVPVLNA	QGLTIAALNC	SSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	
Lib_20	QQDN LRVSPYGMHL	GNRLPAHAQA	TGKVLLSVLD	REVQIEWIEK	YGLKRLTPYT	ITDEHTFLET	LDAVRQSDYC	LTEENELGT	IAIAVPVLNA	QGLTIAALNC	PSQTNRVQPQ	YLIDQVLP LL	RNTANELRNL V	

56 **Figure S1. Amino acid sequence alignment of PobR-WT and 20 randomly selected clones from the PobR mutant library.** Multiple sequence
57 alignment was performed using MAFFT with PobR-WT (271 aa) as the reference sequence. WT indicates wild-type PobR, and Lib_1–Lib_20 indicate
58 20 randomly selected clones from the PobR mutant library. Amino acid residues that differ from PobR-WT are highlighted. Red indicates
59 substitutions at the nine targeted hotspot positions (S118, V143, M148, T159, S160, S212, H216, L220, and M241), gray indicates substitutions at
60 other non-targeted positions, and yellow indicates the fixed deletion at L141 introduced during library construction.

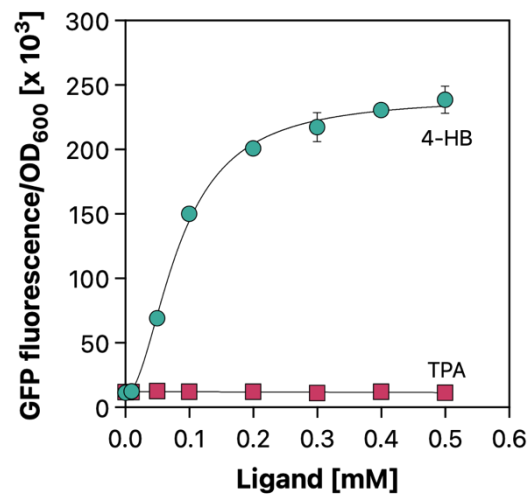


Figure S2. Dose-response analysis of PobR_2 toward 4-HB and TPA over the 0-0.5 mM range.

The green circles were indicated fluorescence intensity of PobR_1 cell for 4-HB, the red squares were indicated fluorescence intensity of PobR_1 cell for TPA. Error bars indicate the standard deviation from two biological replicates. Each biological replicate was prepared from an independently inoculated culture and processed separately.

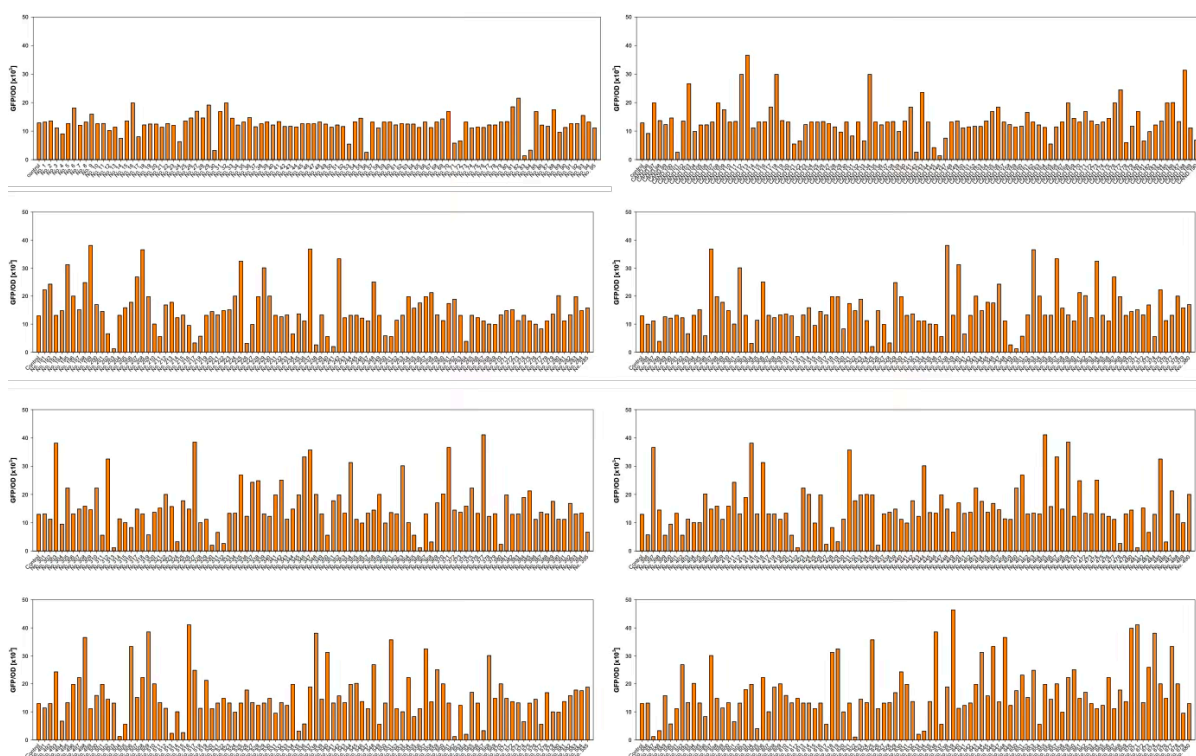


Figure S3. Fluorescence screening of 1,000 single clones recovered after FACS enrichment under 1 mM TPA induction. Recovered clones were individually cultured and analyzed for GFP output to identify variants with increased TPA-responsive fluorescence.

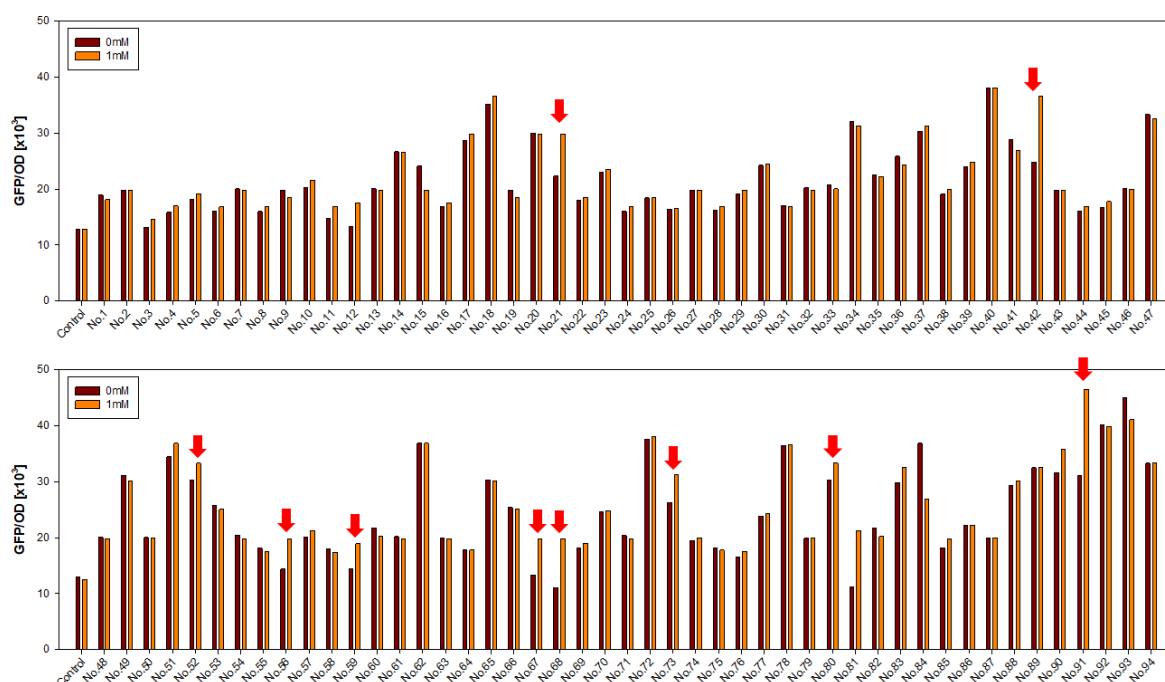


Figure S4. Secondary screening of recovered PobR variants in the absence and presence of 1 mM TPA. Recovered variants were cultured with or without 1 mM TPA, and GFP fluorescence normalized by cell density was measured to identify TPA-responsive clones. Arrows indicate selected variants showing increased TPA-dependent activation.



Figure S6. Sequence logo analysis of amino acid distributions at mutagenized PobR positions recovered during TPA-responsive evolution. Sequence logo representation of amino acid distributions at the eight displayed mutagenized positions (S118, M148, T159, S160, S212, H216, L220, and M241) identified across PobR variants (v1–v10) during directed evolution toward TPA responsiveness. Letter height is proportional to the frequency of each amino acid at the corresponding position among the recovered variants

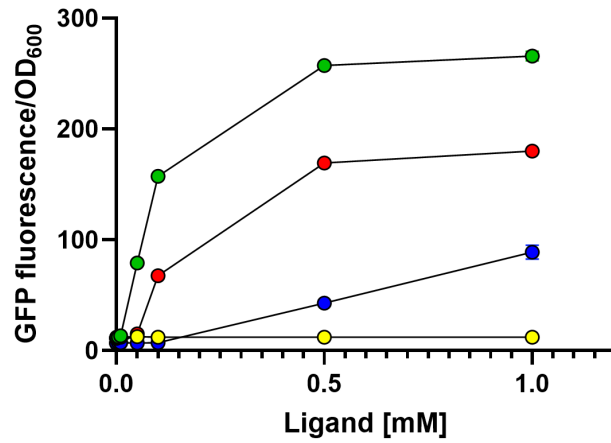


Figure S7. Dose-response comparison of PobR_2 and PobR_3 toward 4-HB and TPA over the 0-1.0 mM range. Green circles indicate the fluorescence intensity of PobR_2 in response to 4-HB, and yellow circles indicate the fluorescence intensity of PobR_2 in response to TPA. Blue circles indicate the fluorescence intensity of PobR_3 in response to 4-HB, whereas pink circles indicate the fluorescence intensity of PobR_3 in response to TPA. Error bars indicate the standard deviation from two biological replicates. Each biological replicate was prepared from an independently inoculated culture and processed separately.

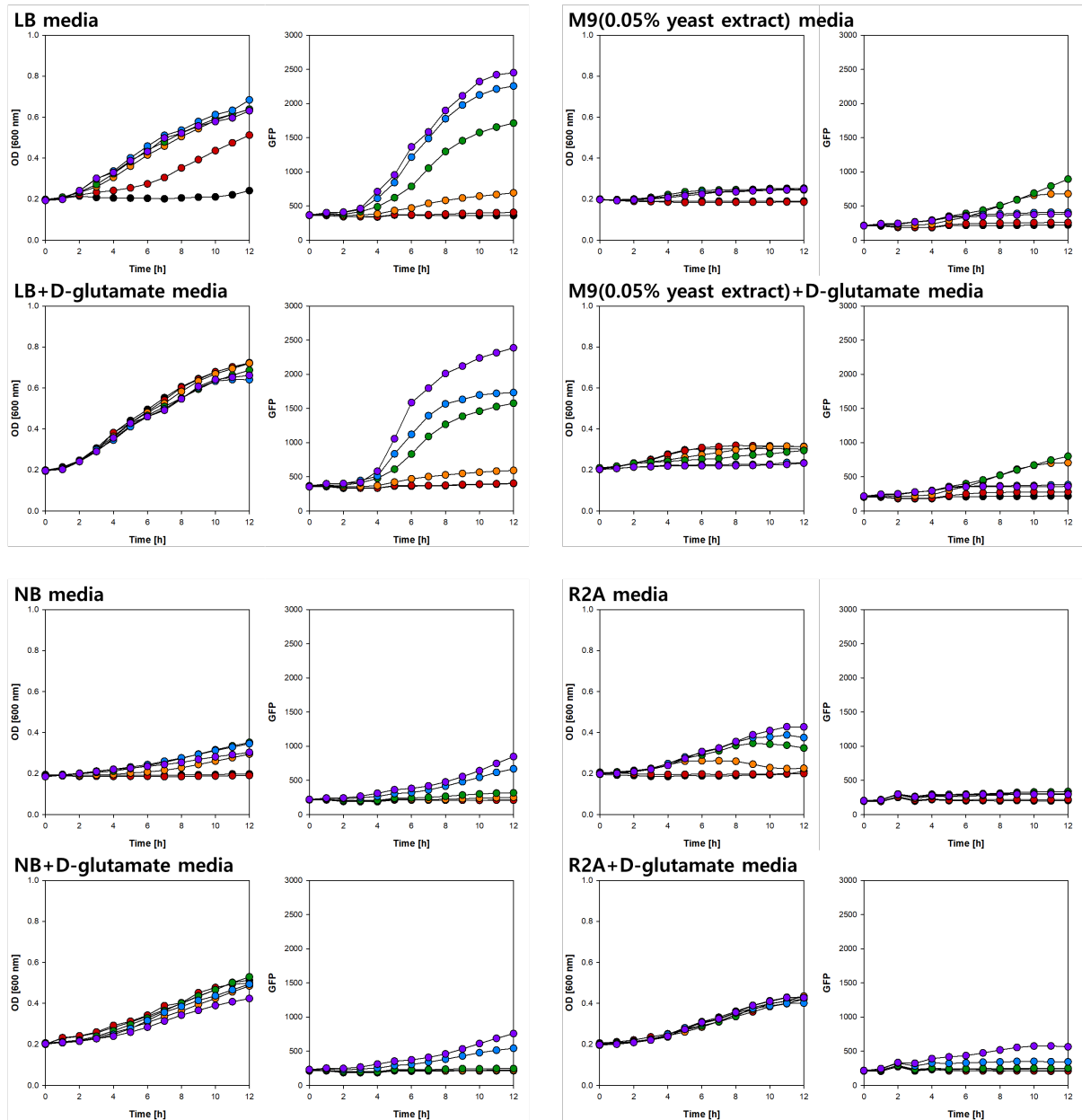


Figure S8. Growth curves and fluorescence outputs of PobR_4 in different media. PobR_4 cells were grown in LB, NB, M9, and R2A media with or without D-glutamate. Black, red, yellow, green, blue, and purple circles indicate TPA concentrations of 0, 0.01, 0.05, 0.1, 0.5, and 1 mM, respectively.

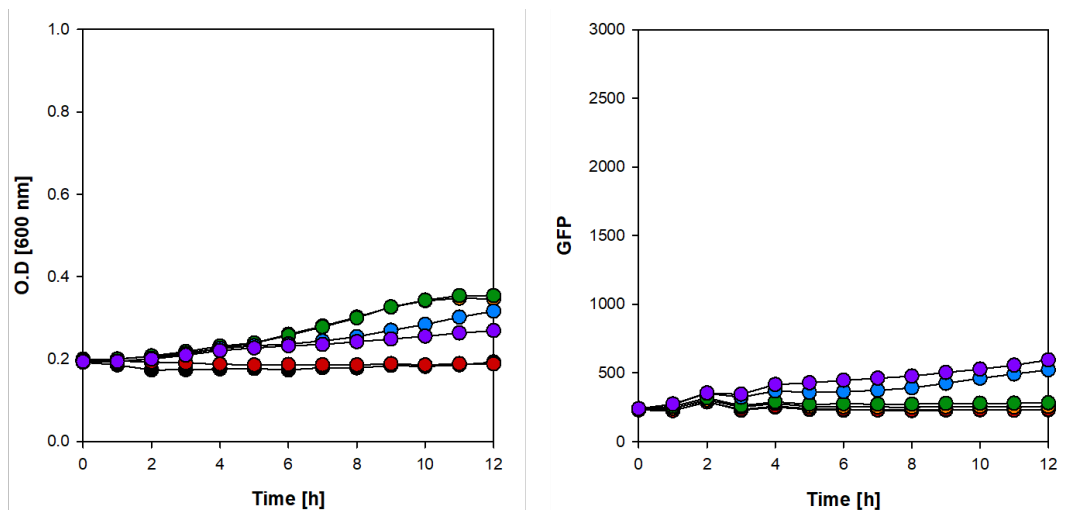


Figure S9. Growth curves and fluorescence outputs of PobR_4 in 1/10-diluted LB medium.

PobR_4 cells were grown in 1/10-diluted LB medium with or without D-glutamate. Black, red, yellow, green, blue, and purple circles indicate TPA concentrations of 0, 0.01, 0.05, 0.1, 0.5, and 1 mM, respectively.

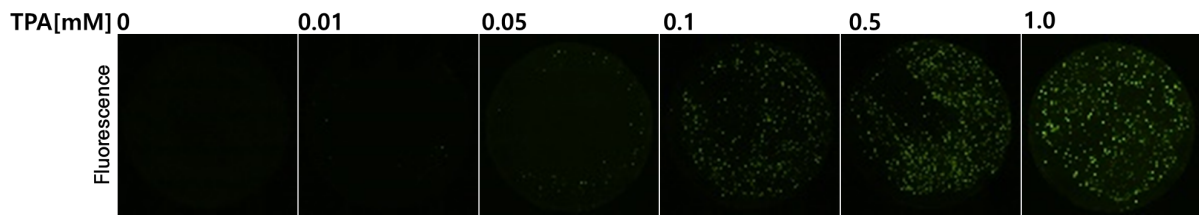


Figure S10. Colony formation and fluorescence of PobR_4 on solid LB agar containing increasing TPA concentrations. PobR_4 cells were plated on LB agar without D-glutamate and supplemented with the indicated concentrations of TPA. Colony formation and fluorescence increased in a TPA concentration-dependent manner.

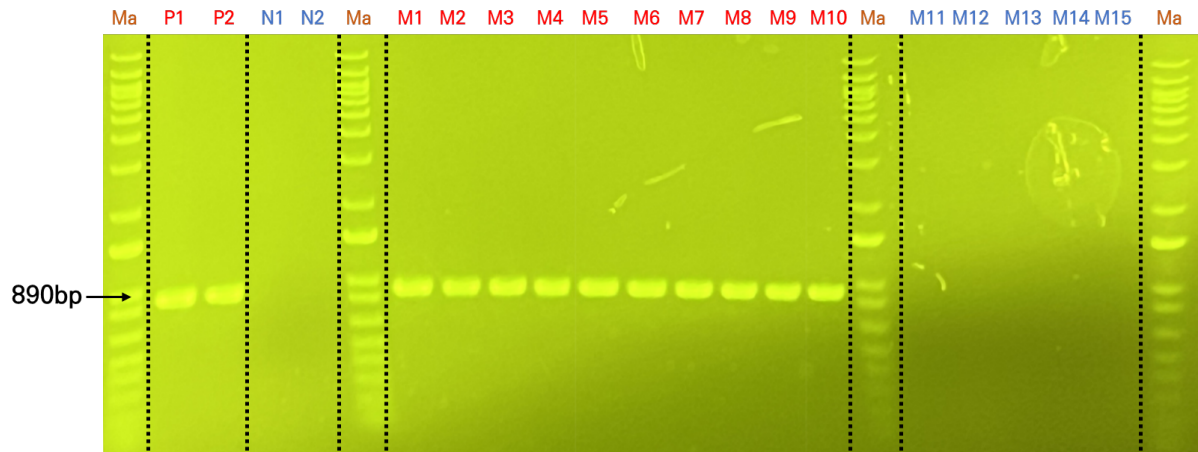


Figure S11. Colony PCR analysis of colonies recovered from the proof-of-concept screening assay. P1 and P2 were selected from positive-control plates, N1 and N2 were selected from negative-control plates, and M1–M15 were selected from mixed plates. The expected 890-bp lcc amplicon was detected in colonies associated with surrounding biosensor fluorescence, confirming the presence of the LCC expression construct.

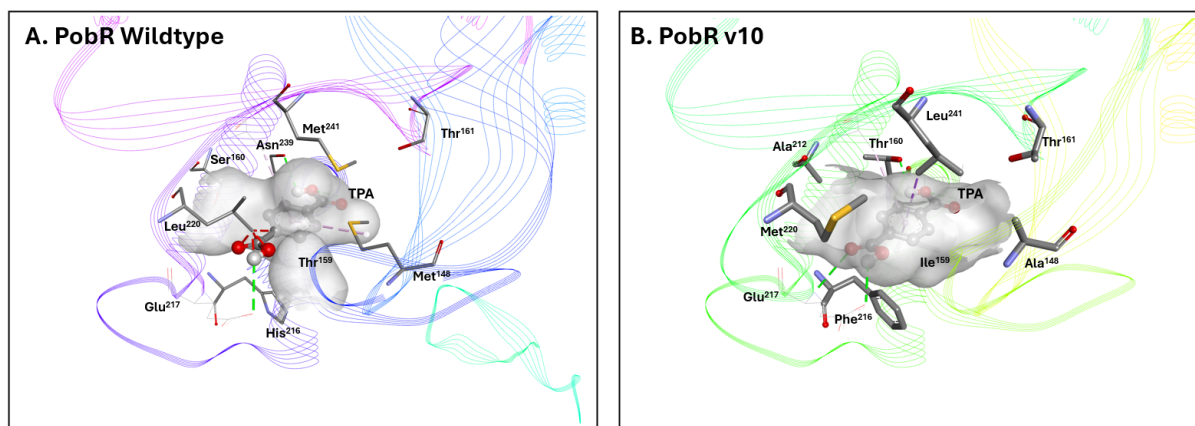


Figure S12. Structural comparison of the predicted ligand-binding pockets of wild-type PobR and PobR_10 with docked TPA. (A) Predicted ligand-binding pocket of wild-type PobR with TPA mapped onto the model. In the wild-type configuration, Met148 and Leu220 are predicted to create steric constraints near the pocket interior, potentially limiting productive accommodation of TPA. Red dashed lines indicate predicted steric constraints. Glu217 is positioned away from the distal carboxylate group of TPA in this configuration. (B) Predicted ligand-binding pocket of PobR_10 with docked TPA. The surface representation suggests an expanded local pocket geometry relative to wild-type PobR, which may help accommodate the distal carboxylate group of TPA. Predicted interactions and pocket changes should be interpreted as computational hypotheses rather than experimentally resolved structural features.