SUPPLEMENTARY DATA

Streamlined CRISPR genome engineering in wild-type bacteria using SIBR-Cas

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Supplementary Figures S1 to S10

Supplementary Tables S1 and S2

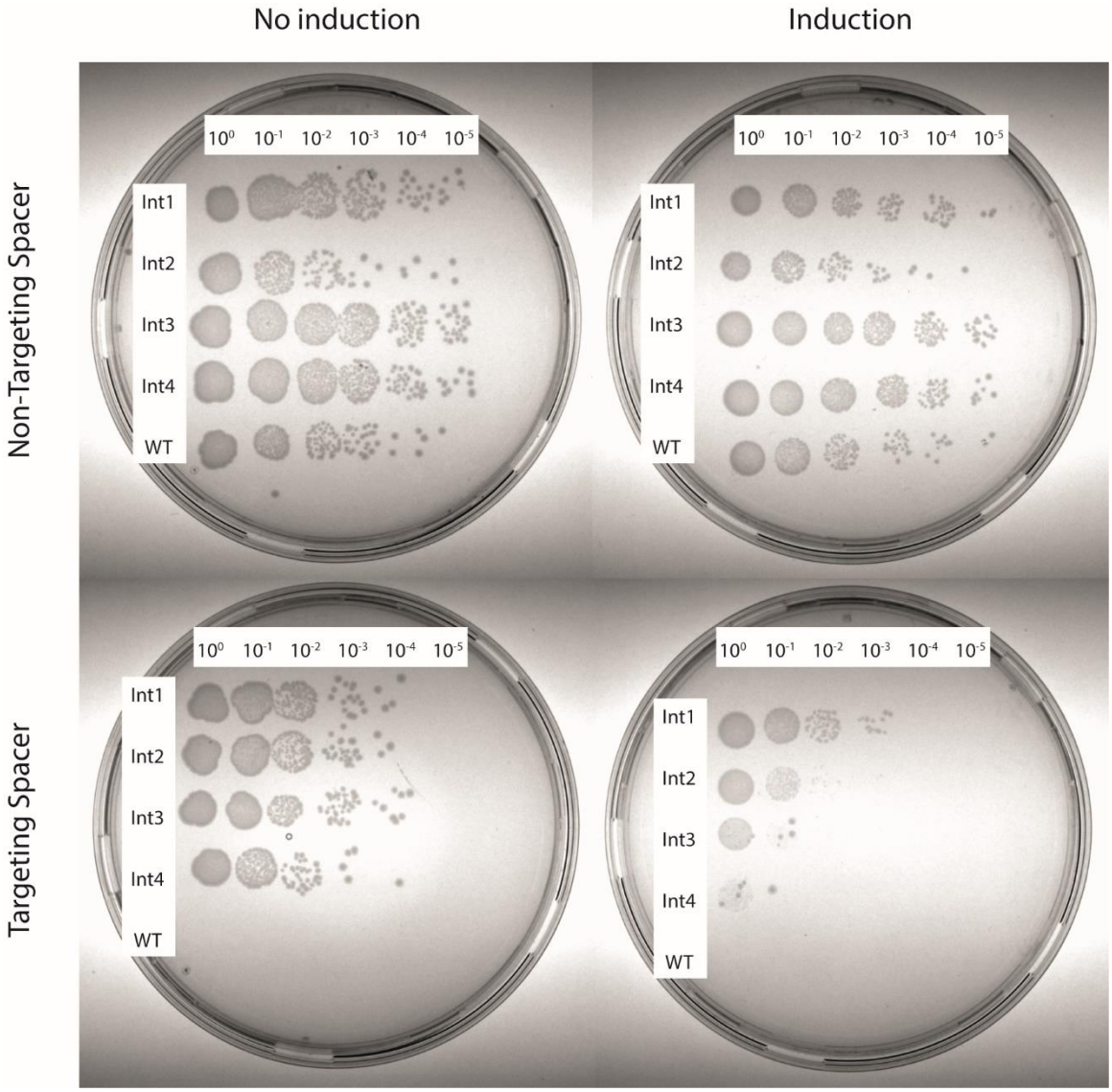

Figure S1. Raw data of SIBR-Cas targeting assay in *E. coli* MG1655. Four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) and the WT-*FnCas12a* were used for counter-selection by targeting *LacZ* with a targeting or a non-targeting spacer (Table S1). Transformants were serially diluted 5 times and plated on LB solid medium containing kanamycin (50 mg L^{-1}) in the presence or absence of the theophylline inducer (2 mM).

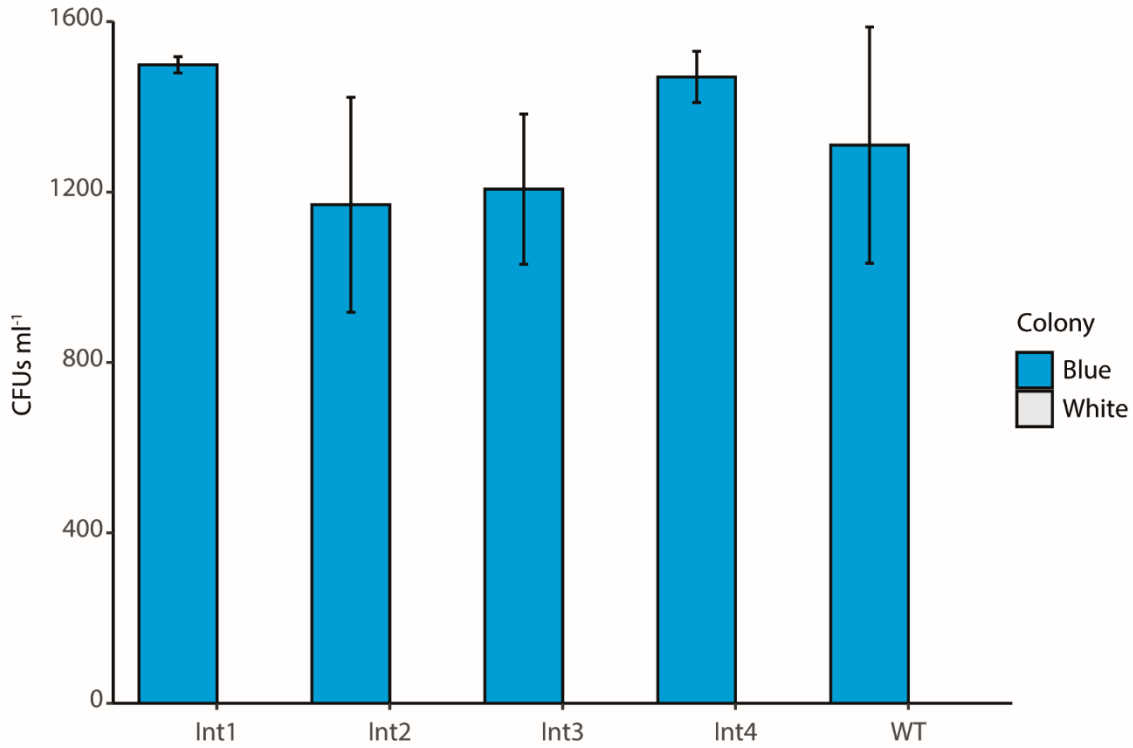

Figure S2. Blue/white colony screening of *E. coli* MG1655 transformed with plasmids containing non-targeting spacers. *E. coli* MG1655 was transformed with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) or the WT-*FnCas12a*, a non-targeting spacer and 500 bp homology arms to facilitate the knock-out of *LacZ* (Table S1). Values and error bars represent the means and s.d. of triplicate experiments.

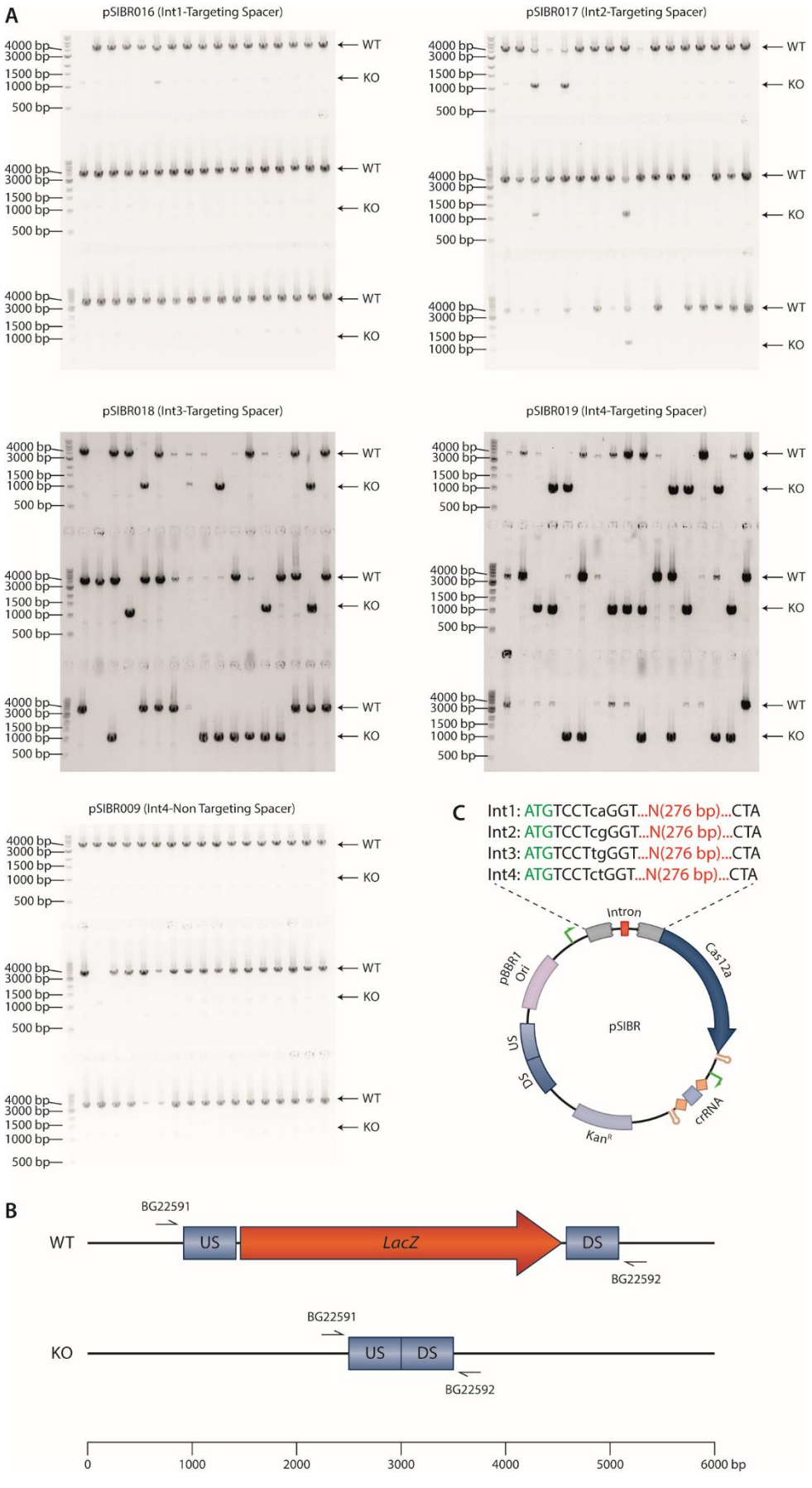
A
pSIBR016 (Int1-Targeting Spacer)
pSIBR017 (Int2-Targeting Spacer)
pSIBR018 (Int3-Targeting Spacer)
pSIBR019 (Int4-Targeting Spacer)
pSIBR009 (Int4-Non Targeting Spacer)
4000 bp
3000 bp
1500 bp
1000 bp
500 bp
WT
KO
C
Int1: ATGTCCTcaGGT...N(276 bp)...CTA
Int2: ATGTCCTcgGGT...N(276 bp)...CTA
Int3: ATGTCCTtgGGT...N(276 bp)...CTA
Int4: ATGTCCTctGGT...N(276 bp)...CTA
Intron
Cas12a
pBBR1 Ori
pSIBR
US
DS
crRNA
Kan^R
B
BG22591
WT
US
LacZ
DS
BG22592
BG22591
KO
US
DS
BG22592
0
1000
2000
3000
4000
5000
6000 bp

Figure S3. *LacZ* knock-out using SIBR-Cas. (**A**) *E. coli* MG1655 was transformed with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) or the WT-*FnCas12a*, a *LacZ* targeting or a non-targeting spacer and 500 bp homology arms to facilitate the knock-out of *LacZ* (Table S1). Each variant was performed in triplicate and 16 colonies (if present) were randomly selected from each replicate for colony PCR. The WT-*FnCas12a* variant targeting *LacZ* did not yield any colonies. The non-targeting variants were all WT and for this reason only Int4 is shown as a representative. Mix amplicons (WT and knock-out bands) were not counted for the total knock-out efficiency percentage. WT: 4229 bp, Knock-out (KO): 1154 bp. (**B**) Schematic representation of WT or edited (KO) *E. coli* MG1655 genome at the *LacZ* locus. BG22591 and BG22592 represent the primers used for PCR (Table S2). (**C**) Schematic representation of the pSIBR plasmids used to knock-out *LacZ*. Int1-4 represent the four different SIBR-Cas variants and their sequence difference is depicted with lowercase nucleotide letters.

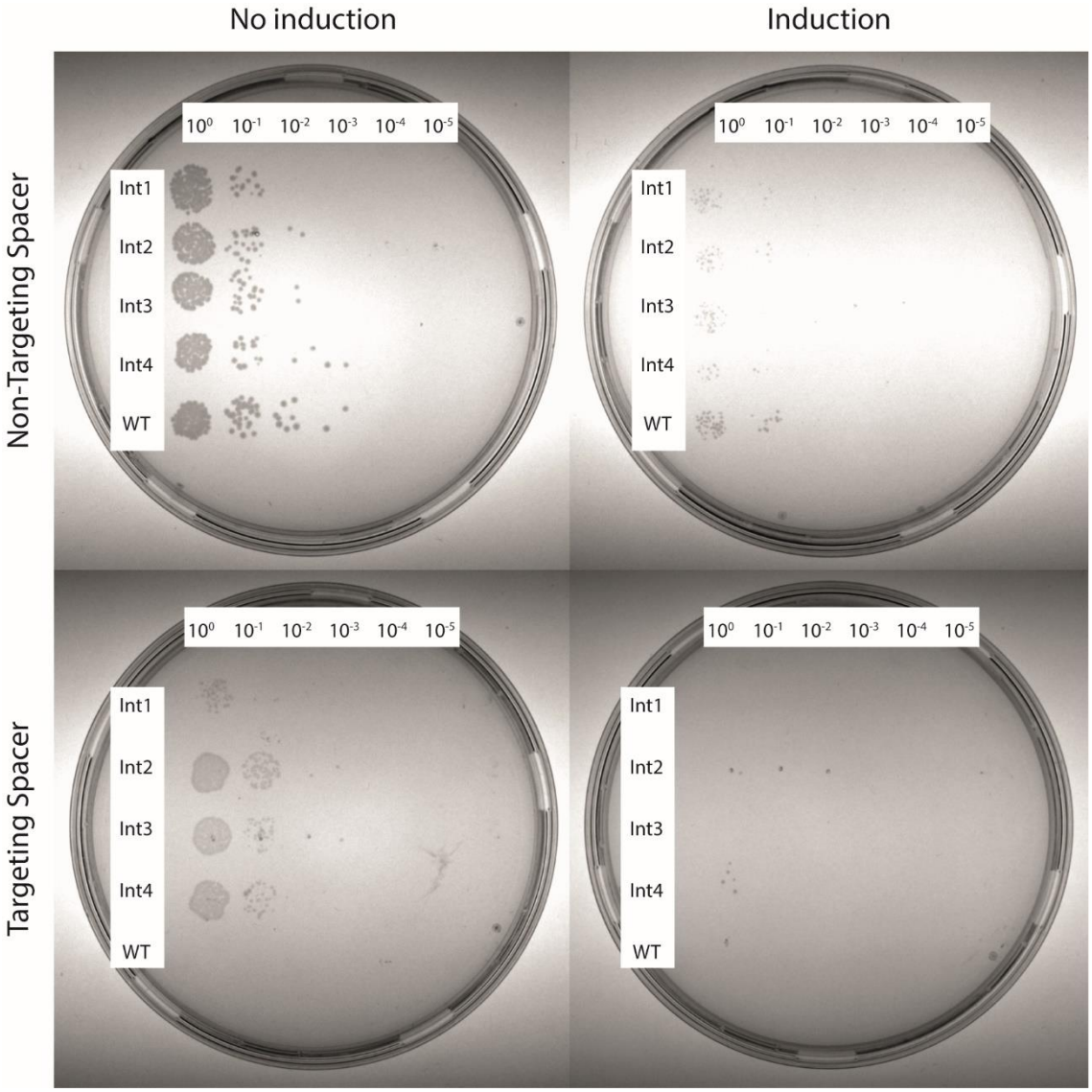

Figure S4. Raw data of SIBR-Cas targeting assay in *P. putida* KT2440. Either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) or the WT-*FnCas12a* were used for counter-selection by targeting *EndA* with a targeting or a non-targeting spacer (Table S1). Transformants were serially diluted 5 times and plated on LB medium containing kanamycin (50 mg L^{-1}) in the presence or absence of the theophylline inducer (2 mM).

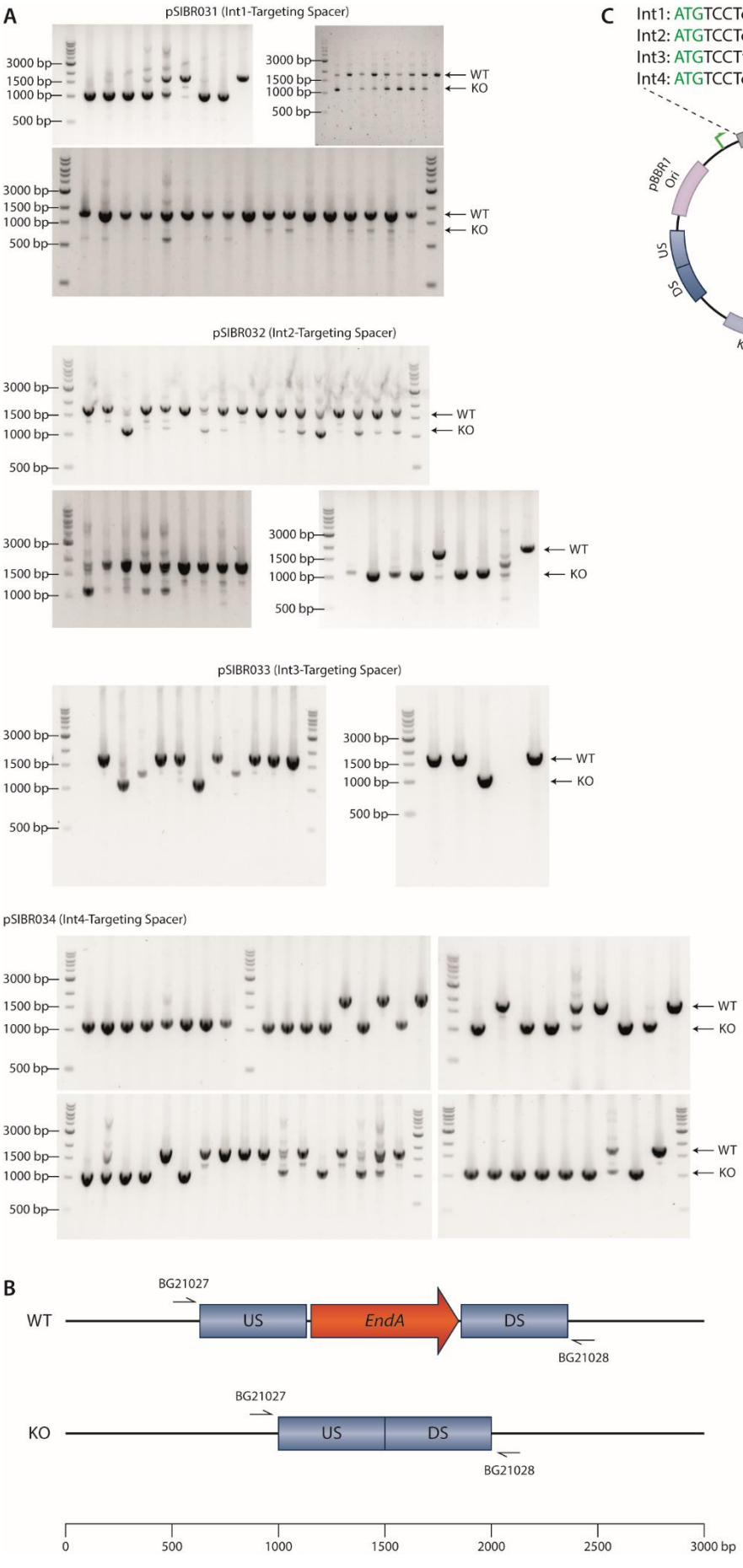
A
pSIBR031 (Int1-Targeting Spacer)
3000 bp
1500 bp
1000 bp
500 bp
WT
KO
pSIBR032 (Int2-Targeting Spacer)
pSIBR033 (Int3-Targeting Spacer)
pSIBR034 (Int4-Targeting Spacer)
C
Int1: ATGTCCTcaGGT...N(276 bp)...CTA
Int2: ATGTCCTcgGGT...N(276 bp)...CTA
Int3: ATGTCCTtgGGT...N(276 bp)...CTA
Int4: ATGTCCTctGGT...N(276 bp)...CTA
Intron
Cas12a
pBBR1 Ori
pSIBR
US
DS
crRNA
KanR
B
BG21027
WT
US
EndA
DS
BG21028
KO
0
500
1000
1500
2000
2500
3000 bp

Figure S5. *EndA* knock-out using SIBR-Cas. (**A**) *P. putida* KT2440 was transformed with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) or the WT-*FnCas12a*, an *EndA* targeting spacer and 500 bp homology arms to facilitate the knock-out of *EndA* (Table S1). Each variant was performed in triplicate and 16 colonies (if present) were randomly selected from each replicate for colony PCR. The WT-*FnCas12a* variant targeting *EndA* did not yield any colonies. Mix amplicons (WT and knock-out bands) were not counted for the total knock-out efficiency percentage. WT: 1814 bp, Knock-out (KO): 1121 bp. (**B**) Schematic representation of WT or edited (KO) *P. putida* KT2440 genome at the *EndA* locus. BG21027 and BG21028 represent the primers used for PCR (Table S2). (**C**) Schematic representation of the pSIBR plasmids used to knock-out *EndA*. Int1-4 represent the four different SIBR-Cas variants and their sequence difference is depicted with lowercase nucleotide letters.

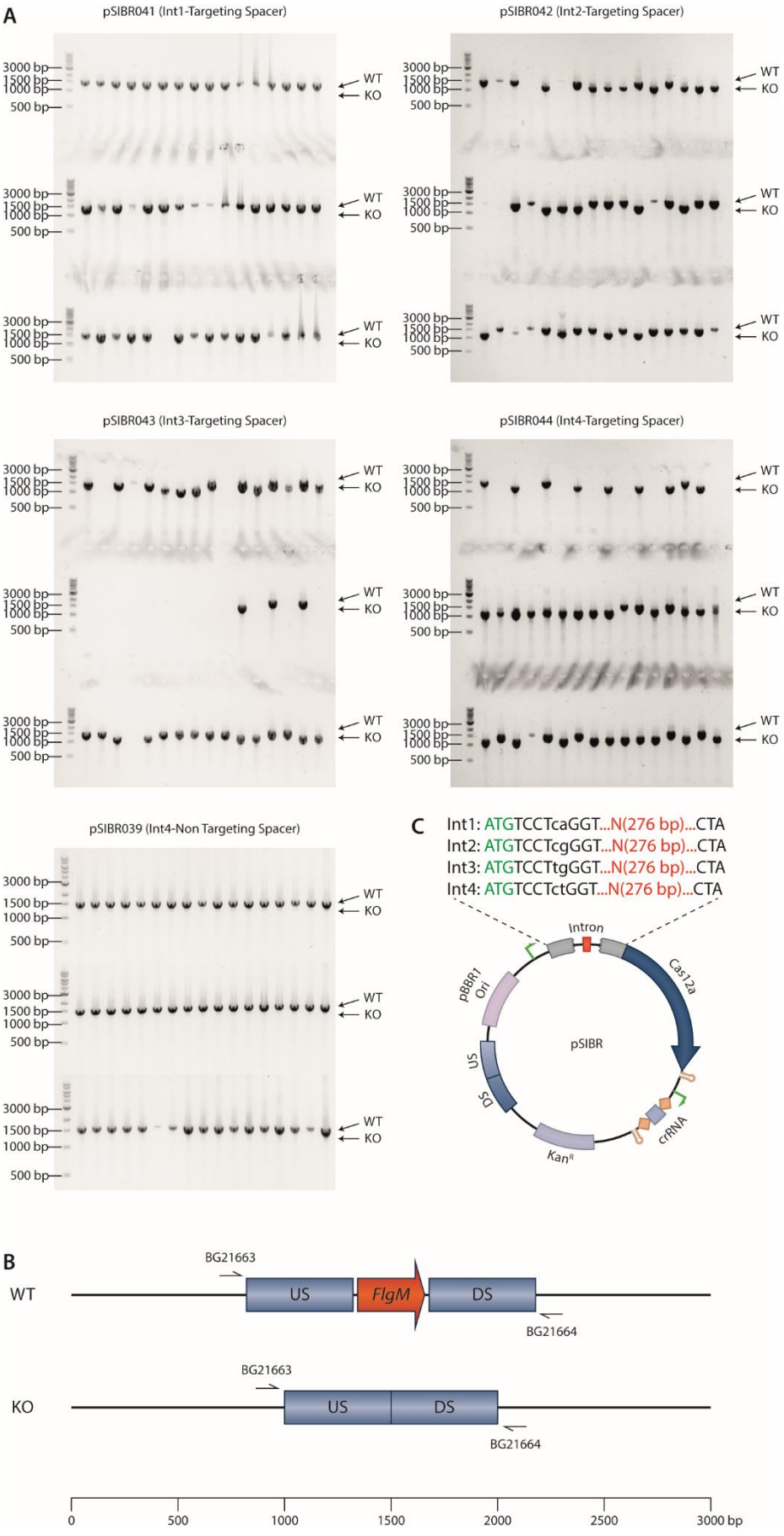
A
pSIBR041 (Int1-Targeting Spacer)
pSIBR042 (Int2-Targeting Spacer)
pSIBR043 (Int3-Targeting Spacer)
pSIBR044 (Int4-Targeting Spacer)
pSIBR039 (Int4-Non Targeting Spacer)
3000 bp
1500 bp
1000 bp
500 bp
WT
KO
C
Int1: ATGTCCTcaGGT...N(276 bp)...CTA
Int2: ATGTCCTcgGGT...N(276 bp)...CTA
Int3: ATGTCCTtgGGT...N(276 bp)...CTA
Int4: ATGTCCTctGGT...N(276 bp)...CTA
Intron
Cas12a
pBBR1 Ori
pSIBR
US
DS
crRNA
Kan^R
B
WT
BG21663
US
FlgM
DS
BG21664
KO
BG21663
US
DS
BG21664
0
500
1000
1500
2000
2500
3000 bp

Figure S6. *FlgM* knock-out using SIBR-Cas. (**A**) *P. putida* KT2440 was transformed with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) or the WT-*FnCas12a*, an *FlgM* targeting or a non-targeting spacer and 500 bp homology arms to facilitate the knock-out of *FlgM* (Table S1). Each variant was performed in triplicate and 16 colonies (if present) were randomly selected from each replicate for colony PCR. The WT-*FnCas12a* variant targeting *FlgM* did not yield any colonies. The non-targeting variants were all WT and for this reason only Int4 is shown as a representative. Mix amplicons (WT and knock-out bands) were not counted for the total knock-out efficiency percentage. WT: 1523 bp, Knock-out (KO): 1208 bp. (**B**) Schematic representation of WT or edited (KO) *P. putida* KT2440 genome at the *FlgM* locus. BG21663 and BG21664 represent the primers used for PCR (Table S2). (**C**) Schematic representation of the pSIBR plasmids used to knock-out *FlgM*. Int1-4 represent the four different SIBR-Cas variants and their sequence difference is depicted with lowercase nucleotide letters.

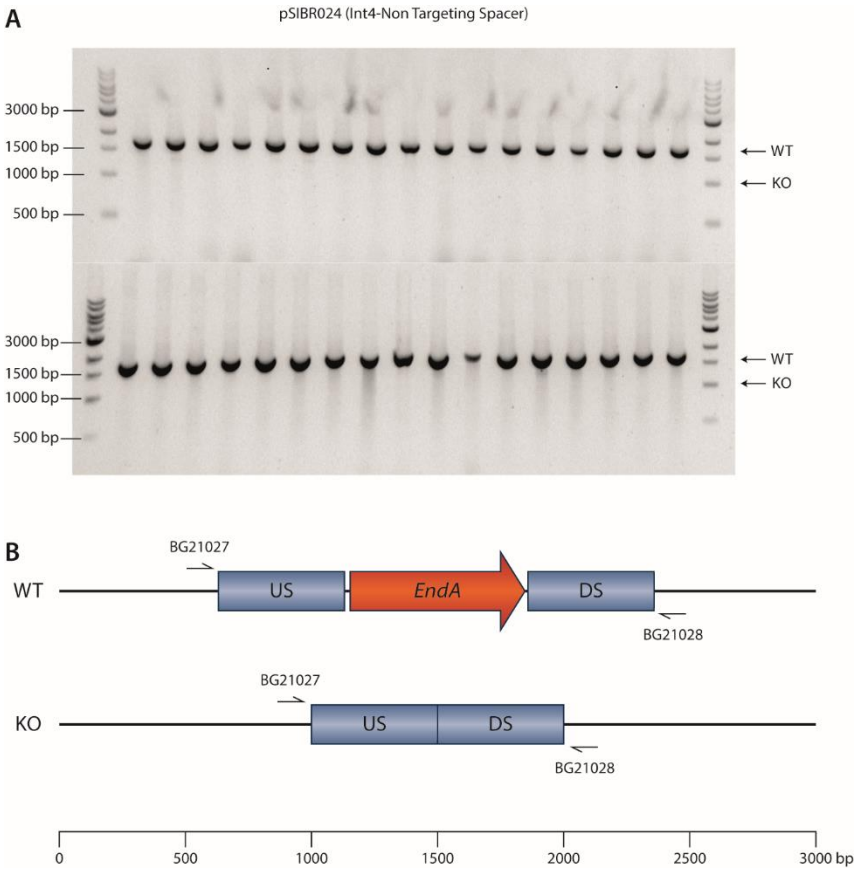

Figure S7. *EndA* knock-out using non-targeting SIBR-Cas. (**A**) *P. putida* KT2440 was transformed with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) or the WT-*FnCas12a*, an *EndA* non-targeting spacer and 500 bp homology arms to facilitate the knock-out of *EndA* (Table S1). Each variant was performed in triplicate and 16 colonies (if present) were randomly selected from each replicate for colony PCR. All variants were WT and for this reason only the Int4 is shown as a representative. WT: 1814 bp, Knock-out (KO): 1121 bp. (**B**) Schematic representation of WT or edited (KO) *P. putida* KT2440 genome at the *EndA* locus. BG21027 and BG21028 represent the primers used for PCR (Table S2).

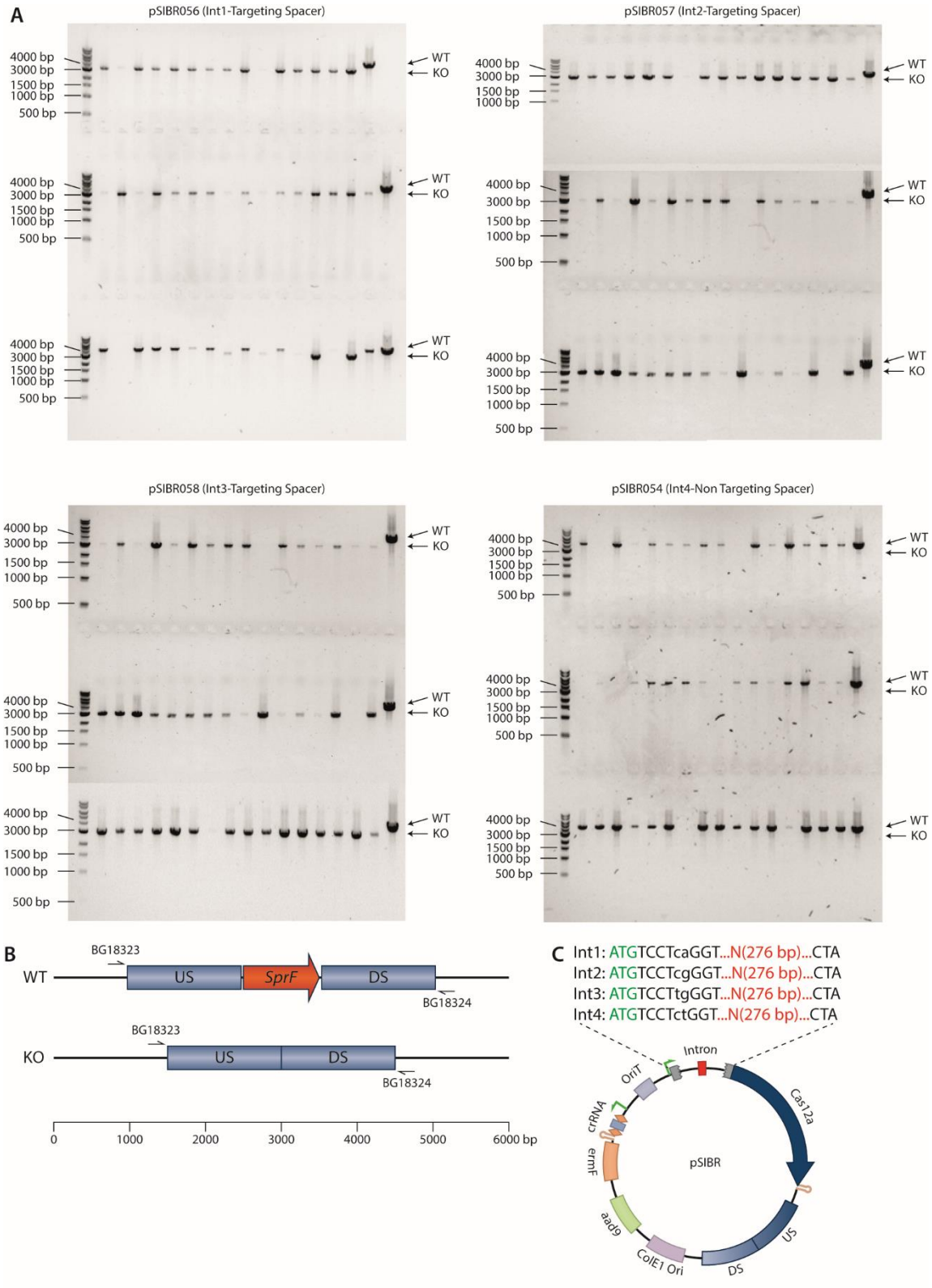

Figure S8. *SprF* knock-out using SIBR-Cas. (**A**) *Flavobaterium* IR1 was transformed with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst

and Int4 is the best splicer) or the WT-*FnCas12a*, an *SprF* targeting or a non-targeting spacer and 1500 bp homology arms to facilitate the knock-out of *SprF* (Table S1). Each variant was performed in triplicate and 16 colonies (if present) were randomly selected from each replicate for colony PCR. The Int4 and WT-*FnCas12a* variants targeting *SprF* did not yield any colonies. The non-targeting variants were all WT and for this reason only Int4 is shown as a representative. Mix amplicons (WT and knock-out bands) were not counted for the total knock-out efficiency percentage. WT: 4081 bp, Knock-out (KO): 3082 bp. (**B**) Schematic representation of WT or edited (KO) *Flavobacterium* IR1 genome at the *SprF* locus. BG18323 and BG18324 represent the primers used for PCR (Table S2). (**C**) Schematic representation of the pSIBR plasmids used to knock-out *SprF*. Int1-4 represent the four different SIBR-Cas variants and their sequence difference is depicted with lowercase nucleotide letters.

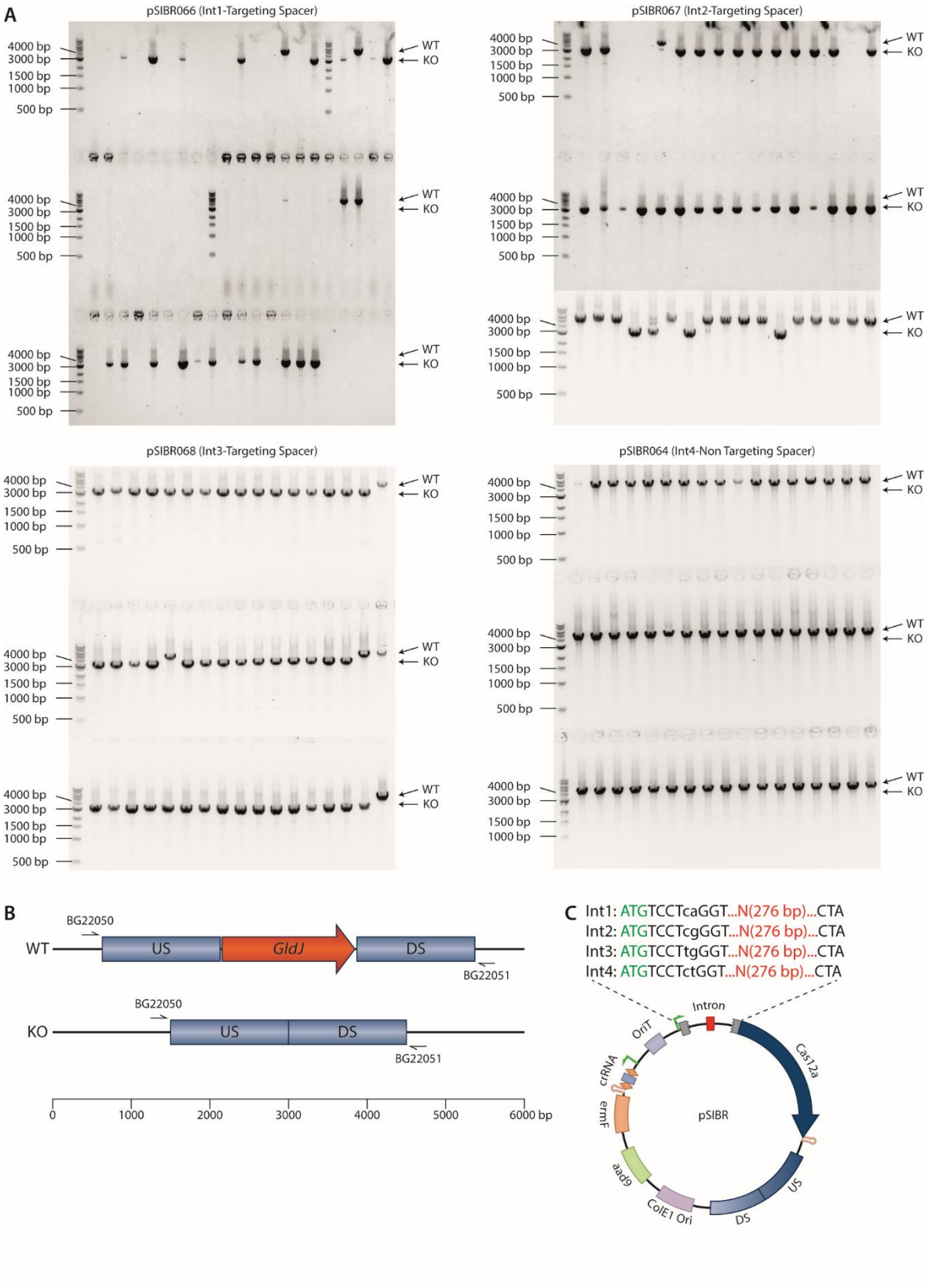

Figure S9. *GldJ* knock-out using SIBR-Cas. (**A**) *Flavobaterium* IR1 was transformed with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst

and Int4 is the best splicer) or the WT-*FnCas12a*, a *GldJ* targeting or a non-targeting spacer and 1500 bp homology arms to facilitate the knock-out of *GldJ* (Table S1). Each variant was performed in triplicate and 16 colonies (if present) were randomly selected from each replicate for colony PCR. The Int4 and WT-*FnCas12a* variants targeting *GldJ* did not yield any colonies. The non-targeting variants were all WT and for this reason only the Int4 is shown as a representative. Mix amplicons (WT and knock-out bands) were not counted for the total knock-out efficiency percentage. WT: 4944 bp, Knock-out (KO): 3255 bp. (**B**) Schematic representation of WT or edited (KO) *Flavobacterium* IR1 genome at the *GldJ* locus. BG22050 and BG22051 represent the primers used for PCR (Table S2). (**C**) Schematic representation of the pSIBR plasmids used to knock-out *GldJ*. Int1-4 represent the four different SIBR-Cas variants and their sequence difference is depicted with lowercase nucleotide letters.

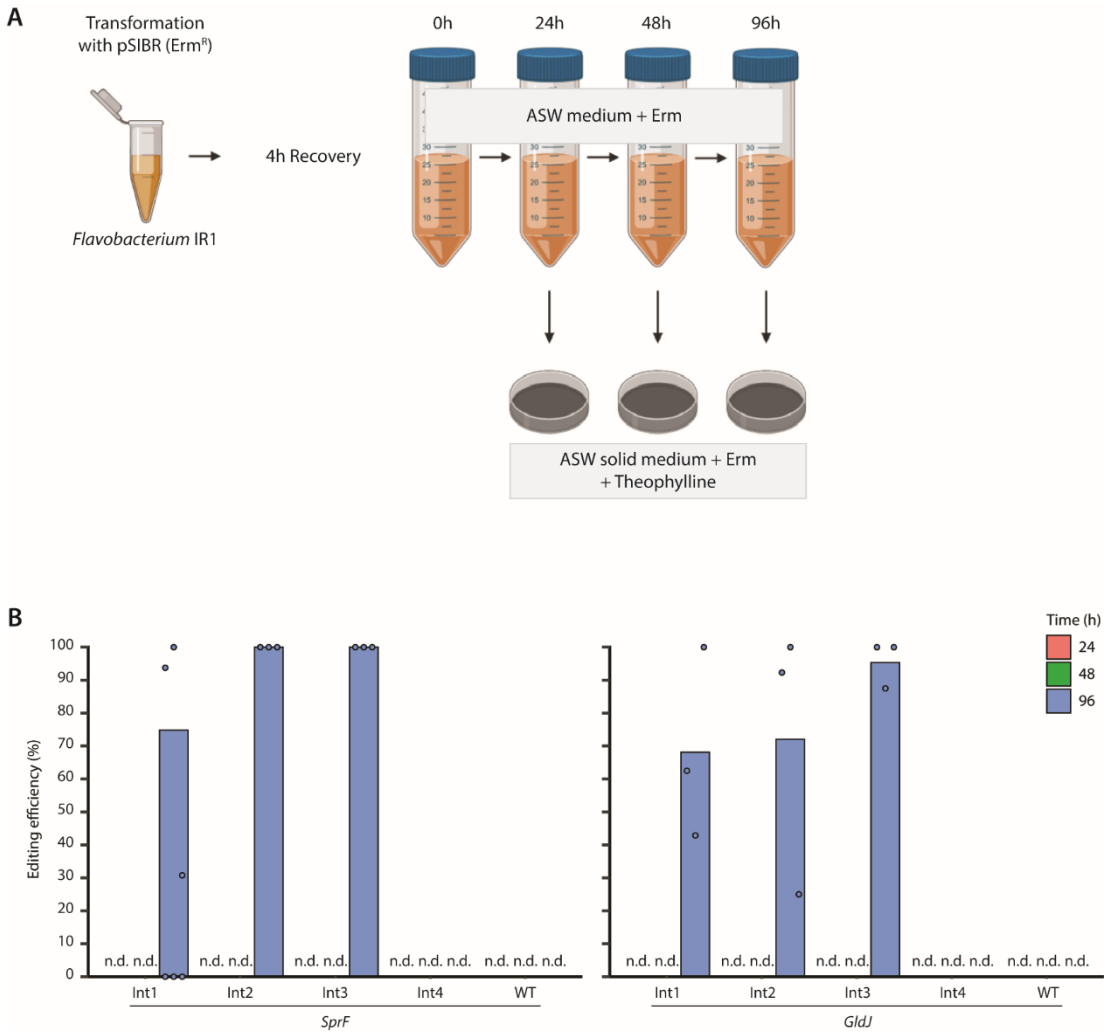

Figure S10. *SprF* and *GldJ* knock-out in *Flavobacterium* IR1 using SIBR-Cas and following a 96 h incubation approach. (**A**) Schematic representation of the 96 h incubation approach. After transformation of *Flavobacterium* IR1 with plasmids containing either of the four different SIBR-Cas variants (Int1-4; Int1 is the worst and Int4 is the best splicer) or the WT-*FnCas12a*, an *SprF* or a *GldJ* targeting spacer and 1500 bp homology arms to facilitate either the knock-out of *SprF* or *GldJ* (Table S1), cells were recovered for 4 hours at 30°C in 1 mL ASW medium. After recovery, the cells were transferred in 10 mL ASW medium containing erythromycin (Erm; 200 mg L^{-1}) and incubated for 96 hours. 1 mL of culture was recovered at 24, 48 and 96 hours of incubation, centrifuged to remove excess medium and plated on ASW agar containing erythromycin (200 mg L^{-1}) and the theophylline inducer (2 mM). This image was created using BioRender.com. (**B**) Editing efficiency of *SprF* and *GldJ* genes in

Flavobacterium IR1 following the 96 h incubation approach as indicated in (A). Colonies were obtained only at 96 hours (blue bars) after transformation and the editing efficiency of the biological triplicates is represented with a blue dot. 24 hours (red bars) and 48 hours (green bars) of incubation did not yield any colonies. Int1 to Int4 represent the four Intron-*FnCas12a* variants and WT represents the WT *FnCas12a* gene. N.d., not determined.

Table S1. Plasmids used in this study.

Plasmid name	Description and relevant characteristics	Reference	Plasmid map (Benchling link)
LacZ assays in *E.coli*			
pEA001 [-7W]	KanR, lacUV5p-[-7W] Intron-LacZ	This study	https://benchling.com/s/seq-b29hHw7mAwS9wCC9XjSh
pEA001 [-7M]	KanR, lacUV5p-[-7M] Intron-LacZ	This study	https://benchling.com/s/seq-b29hHw7mAwS9wCC9XjSh
pEA001 [PPP]	KanR, lacUV5p-PPP Intron-LacZ	This study	https://benchling.com/s/seq-TaewzYsGuJPgQQIUMGWK
pEA001 [PPW]	KanR, lacUV5p-PPW Intron-LacZ	This study	https://benchling.com/s/seq-EOkis573cMPYC19kMe1K
pEA001 [PPM]	KanR, lacUV5p-PPM Intron-LacZ	This study	https://benchling.com/s/seq-WoEO8dYbzWWEfgP4YHZ3
pEA001 [PWP]	KanR, lacUV5p-PWP Intron-LacZ	This study	https://benchling.com/s/seq-v5xeWzkey7xqyDVz049D
pEA001 [PWW]	KanR, lacUV5p-PWW Intron-LacZ	This study	https://benchling.com/s/seq-fyuHmtagQ1frgrXSHLTI
pEA001 [PWM]	KanR, lacUV5p-PWM Intron-LacZ	This study	https://benchling.com/s/seq-FCyiOnKpcH9rjLCkPBuQ
pEA001 [PMP]	KanR, lacUV5p-PMP Intron-LacZ	This study	https://benchling.com/s/seq-lTxfhMjjvaKJqg160zNT
pEA001 [PMW]	KanR, lacUV5p-PMW Intron-LacZ	This study	https://benchling.com/s/seq-JPwy5FGBvEskE2GVbv7P
pEA001 [PMM]	KanR, lacUV5p-PMM Intron-LacZ	This study	https://benchling.com/s/seq-x3TLoIbNNgfV2YQilNRY
pEA001 [MPP]	KanR, lacUV5p-MPP Intron-LacZ	This study	https://benchling.com/s/seq-0XyIkf8SqsFB5t8M1R17
pEA001 [MPW]	KanR, lacUV5p-MPW Intron-LacZ	This study	https://benchling.com/s/seq-6WHDElHCNkgJwg9QMgko
pEA001 [MPM]	KanR, lacUV5p-MPM Intron-LacZ	This study	https://benchling.com/s/seq-8Pv9l5IdfQDM4uE9mEsk
pEA001 [MPW]	KanR, lacUV5p-MPW Intron-LacZ	This study	https://benchling.com/s/seq-6WHDElHCNkgJwg9QMgko
pEA001 [MWW]	KanR, lacUV5p-MWW Intron-LacZ	This study	https://benchling.com/s/seq-o5RmITaeke7KfC26ucN2
pEA001 [MWM]	KanR, lacUV5p-MWM Intron-LacZ	This study	https://benchling.com/s/seq-LXTAIKbOkh0LkUtwS6jN
pEA001 [MMP]	KanR, lacUV5p-MMP Intron-LacZ	This study	https://benchling.com/s/seq-5tcNZAVJGT4y9edfAQ45
pEA001 [MMW]	KanR, lacUV5p-MMW Intron-LacZ	This study	https://benchling.com/s/seq-ZA7JveYYuPSFZYHL6RI4
pEA001 [MMM]	KanR, lacUV5p-MMM Intron-LacZ	This study	https://benchling.com/s/seq-eTGayMzfAzP8rWzB6bGF
pEA001 [296P]	KanR, lacUV5p-[+296P] Intron-LacZ	This study	https://benchling.com/s/seq-r1rCLMW5YYzhYZQMnFu0
pEA001 [296W]	KanR, lacUV5p-[+296W] Intron-LacZ	This study	https://benchling.com/s/seq-7WTrwg0RE4wBvLycHil7
SIBR-Cas targeting and editing in *E. coli* MG1655			
pSIBR001	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t	This study	https://benchling.com/s/seq-6nB5lmpoZV4I4MGsFa08
pSIBR002	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t	This study	https://benchling.com/s/seq-HqcsmCgFky18So0ILu4H
pSIBR003	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t	This study	https://benchling.com/s/seq-ITK2KqCHUltu7RRdTM7z
pSIBR004	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t	This study	https://benchling.com/s/seq-ynlTp7uQfMXeA3ozptCF
pSIBR005	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-NT spacer-B1002t	This study	https://benchling.com/s/seq-oMUyGhlUeozuKBZAw4jc
pSIBR006	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for L*acZ*	This study	https://benchling.com/s/seq-KfOvLlHpPQ3Nc3Letvi2
pSIBR007	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-A9yxFE6tM147TxLnuBp5
pSIBR008	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-GsV7JDjZCptXs74ru8KP
pSIBR009	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-bOnvk6U3KpdodrtOdwqa
pSIBR010	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-UfbGPfRlByNQMPfIRQgf
pSIBR011	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t	This study	https://benchling.com/s/seq-uiQtuVeqvz5zjF7z0ny3
pSIBR012	KanR, lacUV5p-Int2-FnCas12a-B1002t,	This study	https://benchling.com/s/seq-JQKbacCnlyWpi8euHvrn

	lacUV5p-LacZ spacer-B1002t		
pSIBR013	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t	This study	https://benchling.com/s/seq-ylKKtgjKF6DlFbcDwHCQ
pSIBR014	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t	This study	https://benchling.com/s/seq-fkln1V3COjxwQkzR99Tn
pSIBR015	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t	This study	https://benchling.com/s/seq-uT1YvCJDtZK0T3ZV9TSZ
pSIBR016	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t, 500 bp homologous arms for L*acZ*	This study	https://benchling.com/s/seq-2zEd9FIQA0I1iAMMtng9
pSIBR017	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-7KxCoxBOjFf1l5JKPY0V
pSIBR018	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-uyyUS1G1D8LGq0CXwdlO
pSIBR019	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-fAPkx41fFWhXfMxP33Bp
pSIBR020	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-LacZ spacer-B1002t, 500 bp homologous arms for LacZ	This study	https://benchling.com/s/seq-HL3kNsN2thXzkaSbBCuY
SIBR-Cas targeting and editing in *P. putida* KT2440			
pSIBR021	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-SxuPs17CAlx8DACfcRHC
pSIBR022	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-3q2GgUwX70pVGd86YoZz
pSIBR023	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-BWRXhXnYy2XmfKUuFNnW
pSIBR024	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-XZnDqxP8dQWu5CSMU41k
pSIBR025	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-NT spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-IYm1KhIuepDFo3EWjPlR
pSIBR026	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t	This study	https://benchling.com/s/seq-dgVlJzUOcRjjp42yGGb1
pSIBR027	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t	This study	https://benchling.com/s/seq-WQU6RFH5WcTmIlg3b5hF
pSIBR028	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t	This study	https://benchling.com/s/seq-ESDD9SEYlnV9PEXrds52
pSIBR029	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t	This study	https://benchling.com/s/seq-NaPP64FzauzJVp63HOJz
pSIBR030	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t	This study	https://benchling.com/s/seq-u2ZsyKxbD4tPqMorvQnJ
pSIBR031	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-bx2nsbDh3fepPVsTN2iY
pSIBR032	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-CdpX42lFfRmfEdZJ9oUG
pSIBR033	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-9R6r5e6hNU6dBaj3EsiT
pSIBR034	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-vfPXGovDiFmGAsHEBT4n
pSIBR035	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-EndA spacer-B1002t, 500 bp homologous arms for EndA	This study	https://benchling.com/s/seq-YRxyMOOAVlX5leF9nxBX
pSIBR036	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-NT-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-gDxNAZRAqfBfgvEKvtlS
pSIBR037	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-NT-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-x4ZSBS8StDdzT1yEifmA
pSIBR038	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-NT-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-JsWtfIN7RWaeK1EqyCv9

pSIBR039	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-NT-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-xeIvlLM9aFbrFN5JGAGs
pSIBR040	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-NT-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-6knJB0dffuipQURdFxqW
pSIBR041	KanR, lacUV5p-Int1-FnCas12a-B1002t, lacUV5p-FlgM spacer-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-Wb4iXW1NIk2iKe2vPhTL
pSIBR042	KanR, lacUV5p-Int2-FnCas12a-B1002t, lacUV5p-FlgM spacer-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-yKGbB2QsknL1JcFwahSF
pSIBR043	KanR, lacUV5p-Int3-FnCas12a-B1002t, lacUV5p-FlgM spacer-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-50Urz54c5xTwjw316Nes
pSIBR044	KanR, lacUV5p-Int4-FnCas12a-B1002t, lacUV5p-FlgM spacer-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-qByXhAStnuawws86f6Ou
pSIBR045	KanR, lacUV5p-WT FnCas12a-B1002t, lacUV5p-FlgM spacer-B1002t, 500 bp homologous arms for FlgM	This study	https://benchling.com/s/seq-P8mK0lPbLhXKnuyQsToC
SIBR-Cas editing in *Flavobacterium* IR1			
pSpyCas9Fb_NT	AmpR (E. coli), ErmR (Flavobacterium), ompAp-SpyCas9, Hup-ransom spacer sgRNA-ompAt	Carrión et al. (2019)	https://benchling.com/s/seq-iBmrky1QUKQRxSTF4aIx
pSIBR046	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int1 FnCas12a-mapt, Hup-NT spacer-ompAt	This study	https://benchling.com/s/seq-tryMFuIMBcQowzpUhUox
pSIBR047	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int2 FnCas12a-mapt, Hup-NT spacer-ompAt	This study	https://benchling.com/s/seq-fuYCpx2KmNQbSW3ruCvt
pSIBR048	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int3 FnCas12a-mapt, Hup-NT spacer-ompAt	This study	https://benchling.com/s/seq-34dQ4N74oKkn6jYkSxoh
pSIBR049	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int4 FnCas12a-mapt, Hup-NT spacer-ompAt	This study	https://benchling.com/s/seq-SnpUmWz3Tb6iEiB1sKcZ
pSIBR050	SpecR (E. coli), ErmR (Flavobacterium), ompAp-WT FnCas12a-mapt, Hup-NT spacer-ompAt	This study	https://benchling.com/s/seq-w5aqQtyTT8YnsNFvnLBf
pSIBR051	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int1 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-dZi1phxSOvgSfNPC0xVI
pSIBR052	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int2 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-IWXgaNozjrxheoaH3GsO
pSIBR053	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int3 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-iOJXCZaLpoLePAGEWyRj
pSIBR054	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int4 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-e67DTZnqKUWgHReK5w9i
pSIBR055	SpecR (E. coli), ErmR (Flavobacterium), ompAp-WT FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-Ef4po4YgCN3MoTmjNk5Z
pSIBR056	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int1 FnCas12a-mapt, Hup-SprF spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-WkoYYG87CQOlaLzXNI4o
pSIBR057	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int2 FnCas12a-mapt, Hup-SprF spacer-ompAt, 1500 bp homologous arms for	This study	https://benchling.com/s/seq-Fu0ho5T6eUO2GZOmiu0Z

	SprF		
pSIBR058	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int3 FnCas12a-mapt, Hup-SprF spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-RljMtyXgvb1x2BflEyaX
pSIBR059	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int4 FnCas12a-mapt, Hup-SprF spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-6Ma0owMHNGMER9Goxp39
pSIBR060	SpecR (E. coli), ErmR (Flavobacterium), ompAp-WT FnCas12a-mapt, Hup-SprF spacer-ompAt, 1500 bp homologous arms for SprF	This study	https://benchling.com/s/seq-xCL5zkoGDjmQqJ4zMpHm
pSIBR061	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int1 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-chB8po62TOoKZWxUDUba
pSIBR062	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int2 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-3YTUB840ckExcZGvba39
pSIBR063	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int3 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-xchTm7PHiocBjwZ9SFMp
pSIBR064	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int4 FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-mBvNACYHsDYyDCxoRYiH
pSIBR065	SpecR (E. coli), ErmR (Flavobacterium), ompAp-WT FnCas12a-mapt, Hup-NT spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-xUQNzfObQqryscOUPDRE
pSIBR066	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int1 FnCas12a-mapt, Hup-GldJ spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-NzQNSVzLjYTyhEV2ZyR2
pSIBR067	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int2 FnCas12a-mapt, Hup-GldJ spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-U85vC5BwueDjpfqOL2oE
pSIBR068	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int3 FnCas12a-mapt, Hup-GldJ spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-HNAiHbFECkxxe3t9PdWk
pSIBR069	SpecR (E. coli), ErmR (Flavobacterium), ompAp-Int4 FnCas12a-mapt, Hup-GldJ spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-WZi2dhVTrALGUiDMmIjs
pSIBR070	SpecR (E. coli), ErmR (Flavobacterium), ompAp-WT FnCas12a-mapt, Hup-GldJ spacer-ompAt, 1500 bp homologous arms for GldJ	This study	https://benchling.com/s/seq-TQHsQtPuiTAqZp0P2XwE

Table S2. Oligonucleotides used in this study. The spacer moieties of the crRNA used in this study are highlighted with red.

Oligo ID	Sequence (5'to 3')	Description
BG22591	CGGCGAGGATGAGTGCACAG	cPCR for LacZ KO in E. coli, forward
BG22592	GGGAAGGCGACTGGAGTGCC	cPCR for LacZ KO in E. coli, reverse
BG21027	CGAAGTGATGGCCAAGCTGGG	cPCR for EndA KO in P. putida, forward
BG21028	CTGGCGATGGTAGCGATGACC	cPCR for EndA KO in P. putida, reverse
BG21663	CGTGGACGGTATTCGTGCCG	cPCR for FlgM KO in P. putida, forward
BG21664	GGTTGCGGGGCATCGGATTC	cPCR for FlgM KO in P. putida, reverse
BG18323	AATAGACGCTTTAGAGCTAC	cPCR for SprF KO in Flavobacterium IR1, forward
BG18324	CTTAGGGCAATAATTAGTGC	cPCR for SprF KO in Flavobacterium IR1, reverse
BG22050	ACAATTCCTGTGTTCGAGGC	cPCR for GldJ KO in Flavobacterium IR1, forward
BG22051	CACAGACAAAAGCTGGAAGG	cPCR for GldJ KO in Flavobacterium IR1, reverse
BG20154	AGAAGACATAGATCAACGTCGTGACTGGGAAAAGTCTATGTC TTCA	LacZ spacer insertion through Golden Gate, forward
BG20155	TGAAGACATAGACTTTTCCCAGTCACGACGTTGATCTATGTCT TCT	LacZ spacer insertion through Golden Gate, reverse
BG20271	AGAAGACATAGATGGCTGGCTACCAGAACAACGGTCTATGTC TTCA	EndA spacer insertion through Golden Gate, forward
BG20272	TGAAGACATAGACCGTTGTTCTGGTAGCCAGCCATCTATGTCT TCT	EndA spacer insertion through Golden Gate, reverse
BG21614	AGAAGACATAGATATTTCGAAGCCCAGCGCTGAGTCTATGTC TTCA	FlgM spacer insertion through Golden Gate, forward
BG21615	TGAAGACATAGACTCAGCGCTGGGCTTCGAAATATCTATGTCT TCT	FlgM spacer insertion through Golden Gate, reverse
BG17871	AGGTCTCATAGATGATATTCTTACCAGGTTATGGTCTAAGAGA CCA	SprF spacer insertion through Golden Gate, forward
BG17872	TGGTCTCTTAGACCATAACCTGGTAAGAATATCATCTATGAGA CCT	SprF spacer insertion through Golden Gate, reverse
BG22036	AGGTCTCATAGATCCCATAGTAAACGTACCTCCGTCTAAGAG ACCA	GldJ spacer insertion through Golden Gate, forward
BG22037	TGGTCTCTTAGACGGAGGTACGTTTACTATGGGATCTATGAGA CCT	GldJ spacer insertion through Golden Gate, reverse
BG20140	AGCTGTTTCCTGTGTGAAAT	Upstream homology arm for LacZ, forward
BG20644	TAACGCTGCCGCGCCGGTAAGGCATCGTTCCCACTGCGAT	Upstream homology arm for LacZ, reverse
BG20643	TTAATTGGACCGCGGTCCGACCAACACAGCCAAACATCCG	Downstream homology arm for LacZ, forward
BG20139	ATTTCACACAGGAAACAGCTTAATAACCGGGCAGGCCATG	Downstream homology arm for LacZ, reverse
BG20266	CGTCTCAGCGCAGTCAATCTTCCTTCG	Upstream homology arm for EndA, forward
BG20267	CGTCTCAGTAGAGCAAAGAGCTGCAGCGGAT	Upstream homology arm for EndA, reverse
BG20264	CGTCTCATCCGAACCAGTAAAAGTGGCGCCG	Downstream homology arm for EndA, forward
BG20265	CGTCTCAGCGCTCCTCAGGCCAGCGTTTGTA	Downstream homology arm for EndA, reverse
BG21610	CGTCTCATCCGGACTTTTCCGCGACGTGGTG	Upstream homology arm for FlgM, forward
BG21611	CGTCTCAACGGGATCAGAAACCTCTGGGTATTTGG	Upstream homology arm for FlgM, reverse
BG21612	CGTCTCACCGTACGGCGCGCTGACTTC	Downstream homology arm for FlgM, forward
BG21613	CGTCTCAGTAGCGACACACGCAAGTAACGGC	Downstream homology arm for FlgM, reverse
BG17933	CCTCGAGATCTCCATGGACGCAACTAGACGTTACCAATGC	Upstream homology arm for SprF, forward
BG17934	CTTAAATGATCCTATTTTTCGTTGGGGCAATCAATTGTTATC	Upstream homology arm for SprF, reverse
BG17935	ACGAAAAATAGGATCATTTAAG	Downstream homology arm for SprF, forward
BG17936	CCTCTAGAGTCGACGTCACGGTAATTTAGTCCAAAATGGC	Downstream homology arm for SprF, reverse
BG21734	AGTTGGGGCCTCGAGATCTCCATGGACGGAACTTAAATTGCTT CCCGG	Upstream homology arm for GldJ, forward
BG21735	TGTTACAATTAAATATATTGACTCATTCTTAGGTGATAAATTT AGG	Upstream homology arm for GldJ, reverse
BG21736	CAATATATTTAATTGTAACAAAAGCCC	Downstream homology arm for GldJ, forward
BG21737	CCGGGGATCCTCTAGAGTCGACGTCACGCCTCGTGAACTGGA	Downstream homology arm for GldJ, reverse

	TTTATC	
BG5039	GATCTTAAGGATGTTTTGTTGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [-7M]
BG5040	TGACTGCAGAATATTAAACGGTAGCATTATGTTCAGATAAGG TCG	Reverse primer to construct WT 3' exonic flanking region of pEA001 series
BG5206	GATCTTAAGGATGTTTTCTCAGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PPP]
BG5207	GATCTTAAGGATGTTTTCTCGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PPW]
BG5208	GATCTTAAGGATGTTTTCTCTGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PPM]
BG5209	GATCTTAAGGATGTTTTCTTAGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PWP]
BG5210	GATCTTAAGGATGTTTTCTTGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PWW]
BG5211	GATCTTAAGGATGTTTTCTTTGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PWM]
BG5212	GATCTTAAGGATGTTTTCTGAGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PMP]
BG5213	GATCTTAAGGATGTTTTCTGGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PMW]
BG5214	GATCTTAAGGATGTTTTCTGTGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [PMM]
BG5215	GATCTTAAGGATGTTTTCCCAGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MPP]
BG5216	GATCTTAAGGATGTTTTCCCGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MPW]
BG5217	GATCTTAAGGATGTTTTCCCTGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MPM]
BG5218	GATCTTAAGGATGTTTTCCTAGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MPW]
BG5219	GATCTTAAGGATGTTTTCCTGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MWW]
BG5220	GATCTTAAGGATGTTTTCCTTGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MWM]
BG5221	GATCTTAAGGATGTTTTCCGAGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MMP]
BG5222	GATCTTAAGGATGTTTTCCGGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MMW]
BG5223	GATCTTAAGGATGTTTTCCGTGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [MMM]
BG5304	GATCTTAAGGATGTTCTTTTGGGTTAATTGAGGCCTGAGTATA AGGTG	Forward primer to construct pEA001 [-7W]
BG5305	TGACTGCAGAATATTAAACGGGAGCATTATGTTCAGATAAGG TCG	Reverse primer to construct pEA001 [296P]
BG5306	TGACTGCAGAATATTAAACGGAAGCATTATGTTCAGATAAGG TCG	Reverse primer to construct pEA001 [296W]