Supplemental materials

Genome editing in *Clostridium saccharoperbutylacetonicum* N1-4 using CRISPR-Cas9 system

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TABLE S1 Primers used in this study

Primers	Sequence (5'-3')
YW484	AAAGTTAAAAGAAGAAAATAGAAATATAATCTTTAATTTGAAAAGATTTAAG
YW1041	TTGCTATTTCTAGCTCTAAAACGTTGTATGAACTGCTCCAGAATGGTGGAATGATAAGGG
YW1035	TTGCTATTTCTAGCTCTAAAACGAACTTATGCATATAGCTCGATGGTGGAATGATAAGGG
YW1036	GAACTTATCCTTAATAGTGTCTTTGTCTCCTCTC
YW1037	GAGAGGAGACAAAGACACTATTAAGGATAAGTTCAT
YW1038	GGAGCATGTTCACCAATTCC
YW1039	TCTTTGTGATATGACTAATAATTAGCGGCCGCCCGATAGCGCATTTGCTCTGCTT
YW1040	TCCACTAGTAACCATCACACTGGCGGCCGCGGAGCATGTTCACCAATTCC
YW1053	TTGCTATTTCTAGCTCTAAAACTCTACTGGCTTAAGCATTCCATGGTGGAATGATAAGGG
YW949	TGATATGACTAATAATTAGCGGCCGCAACAAAGAAAGTGGCAGTG
YW950	TTACTTTAATATTATGTAAATCCCCCTCAATTTA
YW951	CAATTAAATTGAGGGGGATTTACATAATATTAAAGTAAAGGCATTTTGA
YW952	ACTAGTAACCATCACACTGGCGGCCGCGCCTATGAATCAAATAAATTTG
YW880	AGACGCATGGCTTTCAAAAA
YW881	CCGTTTACGAAATTGGAACAG
YW1044	TAGTGCTGTTTAGGGTTATTAAGGC
YW1045	AGTTAATGCTCTTGATCTAATTTCC
YW953	ACGATTTATTAGCAAGATTAAAGGAA
YW954	AGAAAGCCTATCCATCAACT
YW200	CGGCATCAGAGCAGATTGTA
YW847	TTAAGATTTAAAAAGGTTACTATGATAATTCTC
YW1042	AGTTAAAAGAAGAAAATAGAAATAGAAATGGTATAAAGAAGTTATATCTACTAATGGAGC
YW1043	TTGCTATTTCTAGCTCTAAAACGTTGTATGAACTGCTCCAGAGTAAACACCCTCTTTCAC
YW1340	ATTTGACAAAAATGGGCTCGTGTTGTACAATAAATGTTCTGGAGCAGTTCATACAACGTTT TAGAGCTAGAAATAGCAAG
YW1341	AAAGTTAAAAGAAGAAAATAGAAATTTATTAACGTTGATATAATTTAAATTTTATTTGACA AAAATGGGCTCGTGTTGT
YW1342	GGATCCACTAGTAACCATCACACTGGCGGCCGCTAATTATTAGTCATATCACAAAGAAT
YW1338	GCTCAGTCCTAGGTATAATGCTAGCTCTGGAGCAGTTCATACAACGTTTTAGAGCTAGAAA TAGCAAG
YW1339	AAAGTTAAAAGAAGAAAATAGAAATTTGACAGCTAGCTCAGTCCTAGGTATAATGCTAGC

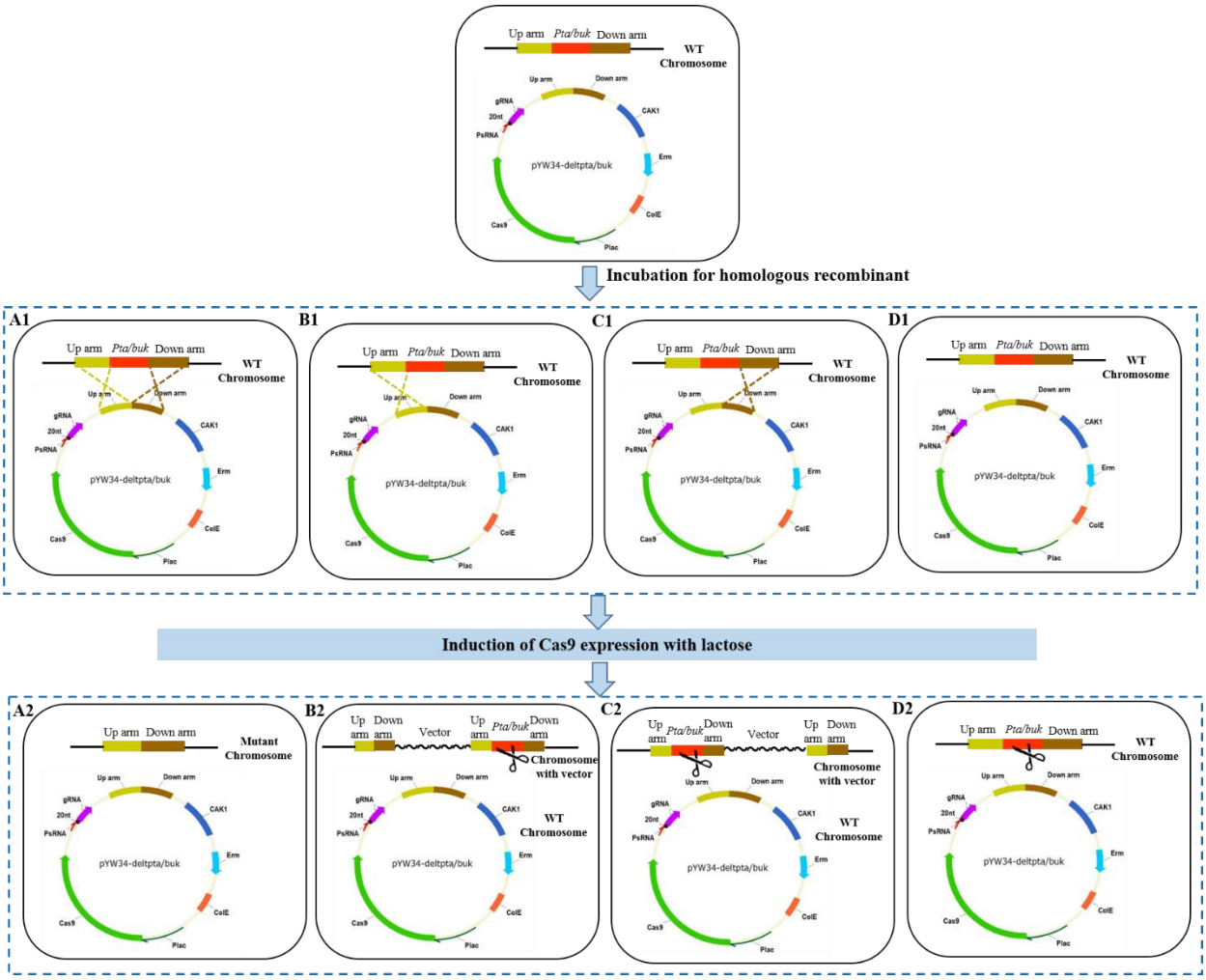

Figure S1. The gene deletion and mutant screening strategy in this study.

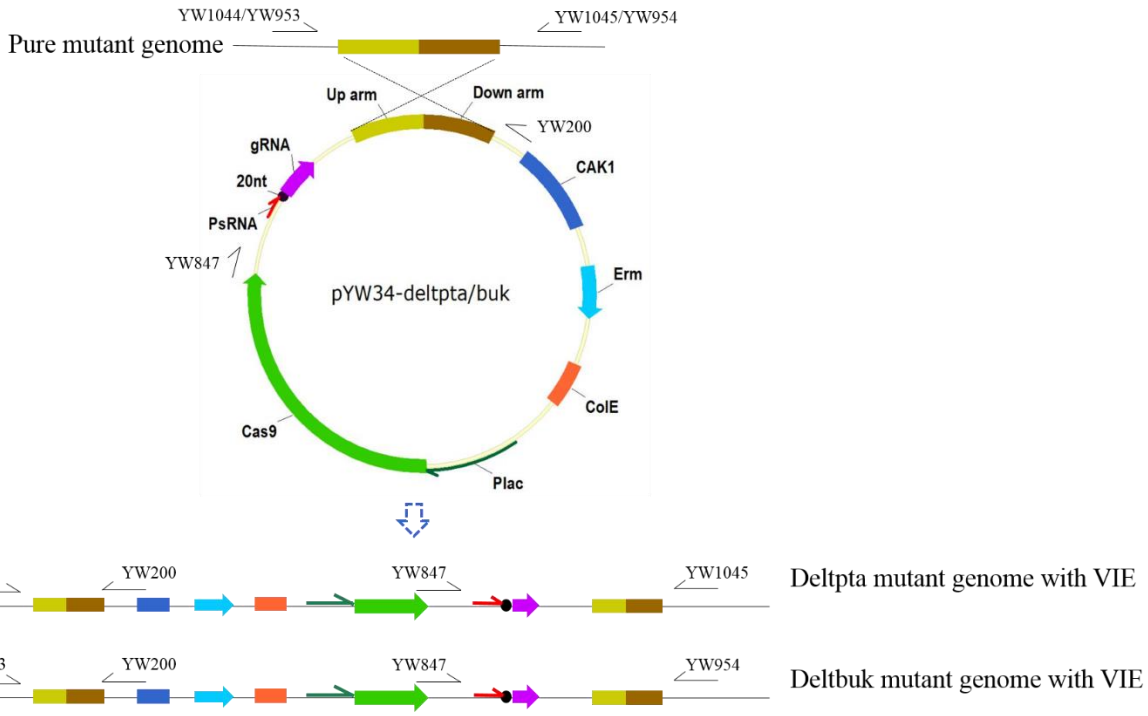

Figure S2. Primers were designed to detect the vector integration event (VIE) during genome editing. As reported previously (1), vector integration occurs due to the additional crossover event between the existing plasmids (YW34-deltpta or pYW34-deltbuk) with the obtained 'pure' mutant, resulting in the mutant containing two copies of the homology arms with the other elements from the vector integrated in the middle (as shown in the figure above). To detect VIE, a pair of primer with one anneals to the plasmid (YW200 for the upstream VIE and YW847 for the downstream VIE) and the other anneals to the chromosome (YW1044/YW1045 for deltpta, YW953/YW954 for deltbuk) were used. In another words, YW1044/YW200 was used to detect the upstream VIE, while YW847/YW1045 was used to detect the downstream VIE during *pta* deletion. YW953/YW200 and YW847/YW954 were used to detect the upstream and downstream VIE during *buk* deletion, respectively. VIE was confirmed by the appearance of PCR band corresponding to the existence of the upstream (2,571 bp for *pta* deletion, 2,318 bp for *buk* deletion) and downstream VIE (2,669 bp for *pta* deletion, and 2,490 bp for *buk* deletion).

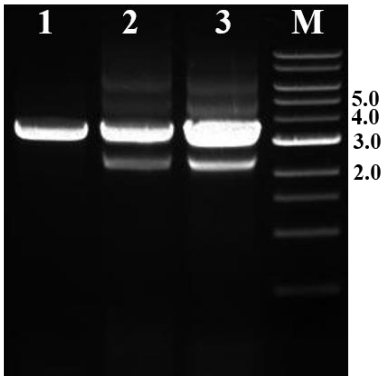

Figure S3. Detection of *pta* deletion using PCR with the TGYLE liquid culture as template. Lane 1, wild type; Lanes 2 and 3, cell transformants subcultured for two generations in the TGYLE medium; Lane M, 1 kb DNA ladder from NEB with numbers on the right representing the band size in kb. Besides the wild type band (3,280 bp), an additional shorter band (2,278 bp) appeared in Lanes 2 and 3 indicating the appearance of the *pta* deletion mutant.

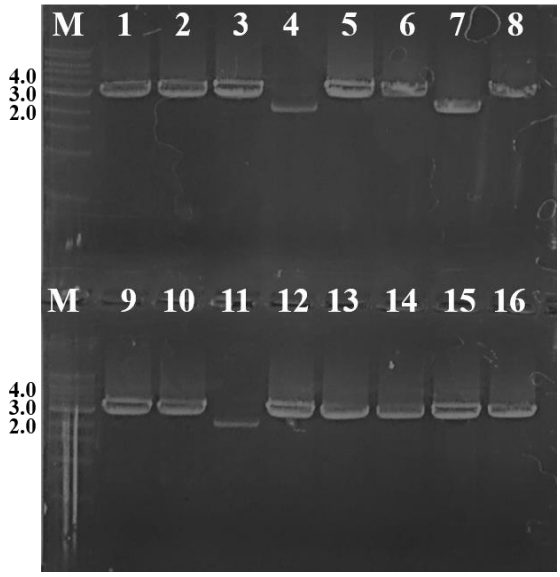

Figure S4. Detection of *pta* deletion in the colonies (replated from the fourth generation of subculturing) using colony PCR (cPCR). Lane M, 1 kb DNA ladder from NEB with numbers on the left representing the band size in kb. Out of the 16 colonies tested, three colonies generated the pure mutant band (2,278 bp for *pta* deleted mutants vs. 3,280 bp for wild type) in Lanes 4, 7 and 11, respectively.

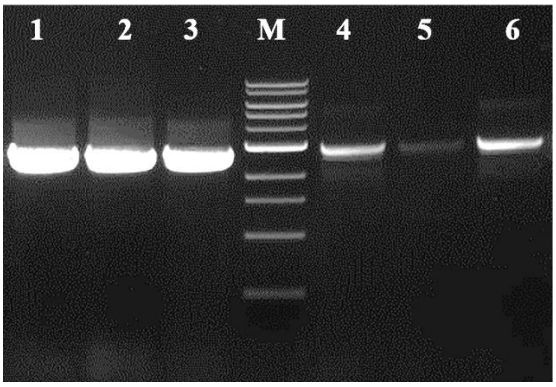

Figure S5. Vector integration event (VIE) detection in the *pta* deletion mutants. Lane 1-3, cPCR results using primers YW1044 and YW200 to detect VIE (2,571 bp) at the up end of the mutant site from the three mutant colonies, respectively, presented in Figure S3. Lane 4-5, cPCR results using primers YW847 and YW1045 to detect VIE (2,669 bp) at the down end of the mutant site from the three mutant colonies, respectively, presented in Figure S3. Lane M, Lane M, 1 kb DNA ladder from NEB with size from up to bottom: 10.0 kb, 8.0 kb, 6.0 kb, 5.0 kb, 4.0 kb, 3.0 kb, 2.0 kb, 1.5 kb, 1.0 kb and 500 bp.

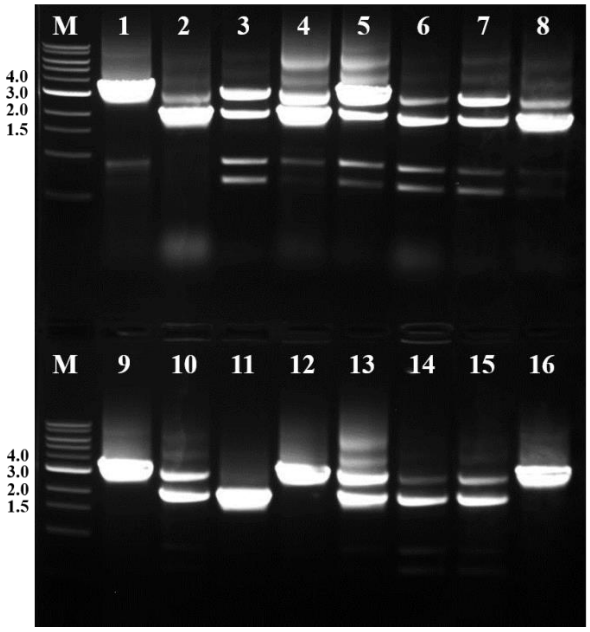

Figure S6. Detection of *buk* deletion in the colonies (replated from the seventh generation of subculturing) using colony PCR (cPCR). Lane M, 1 kb DNA ladder from NEB with numbers on the left representing the band size in kb. Out of sixteen colonies tested, one pure mutant was observed in Lane 11 with only the mutated band (2,061 bp). Double bands (2,061 bp for the mutant and 3,119 bp for the wild type) were detected in eleven colonies represented in Lanes 2-8, 10, 13-15.

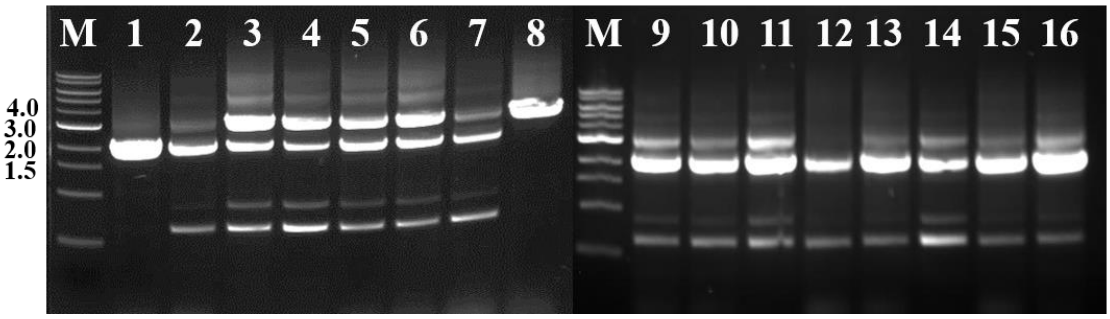

Figure S7. Detection of *buk* deletion in *C. saccharoperbutylacetonicum* deltpta using colony PCR (cPCR). Lane M, 1 kb DNA ladder from NEB with numbers on the left representing the band size in kb. Out of sixteen colonies tested, two pure mutants were observed in Lanes 1 and 12 with only the mutant band (2,061 bp). Except one colony in Lane 8 showed only the wild type band (3,119 bp), the other 13 colonies all demonstrated double bands (2,061 bp for the mutant and 3,119 bp for the wild type).

References

1. **Wang Y, Zhang ZT, Seo SO, Lynn P, Lu T, Jin YS, Blaschek HP.** 2016. Bacterial genome editing with CRISPR-Cas9: deletion, integration, single nucleotide modification, and desirable "clean" mutant selection in *Clostridium beijerinckii* as an example. ACS Synth. Biol.