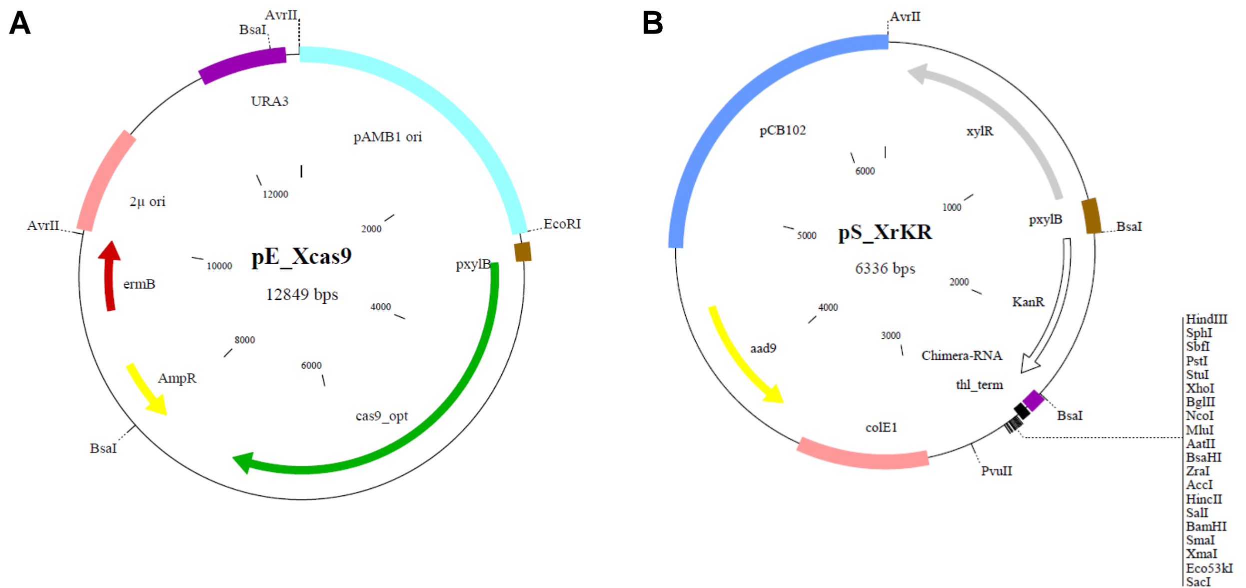
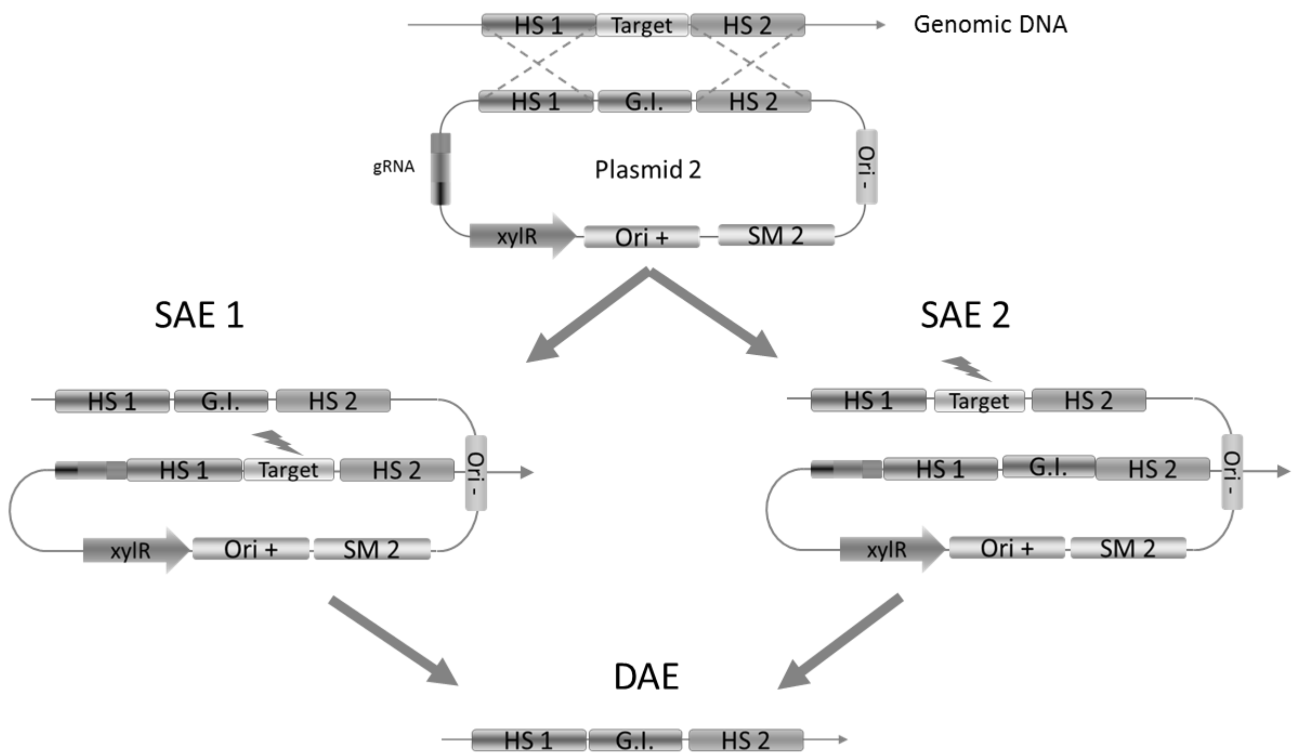
Name	SEQUENCES 5' --> 3'
Construction of pE_Xcas9	
P1_xylB_F	TATTACGCCAGCTGGCGAAAGGGGGATG AAATAAAAGGTTATTTTGCATTGACAAAG
Cas9_xylB_F	GTACTTTTTATCCAT CCCTCCTTGAATGCCACTTCATAACTATCG
xylB_cas9_F	GGCATTCAAGGAGGG ATGGATAAAAAGTACAGTATTGGTCTAG
16-P2_cas9R	GCTGGCCTTTTGCTCACATGTTC TTTCTAAAAAAATTCTTAATCTCCGCCTAGTTGAC
Construction of pS_X^RKR	
P1_xylR_R	TATTACGCCAGCTGGCGAAAGGGGGATG AAATAAAAGGTTATTTTGCATTGACAAAG
bsaI_K_XylB_R	CATAACACCCCTTGTA**GGTCTC** CCTCCTTGAATGCCACTTCATAAC
bsaI_X_kan_F	CATAACACCCCTTGTA**GGTCTC** CCTCCTTGAATGCCACTTCATAAC
bsaI_R_Kan_R	CTAGCTCTAAAACC**GAGACC** CCAATTCTGATTAGAAAAACTC
bsaI_traCRNA_F	AGAATTGG**GGTCTC**GGTTTTAGA GCTAGAAATAGCAAGTTAAAATAAG
MCS_Term_R	AGATCTCGAGGCCTGCAGGCATGCAAGCTTGAATTTTTTTAAAAAATAACTCTGTAG
Construction of pS_X^R_KR_*celA*	
P1_hbd_F	AAAAAATTCAAGCTTGCATGCCTGCAGGCCATTTTAGCTAAGGATATCTATAATG
0326_celA_R	AGTTATTTATATATTTCCAT GATTACGAATTCGAGCTCGGTACCC
celA_0326_F	CGAATTCGTAATCATG GAAATATATAAATAACTACTAGATTACATAAATAAG
P2_0326_R	CGCGTCCATGGAGATCTCGAGGCCTGCAGG AGATGCTCCTGTCTCCTGTATTTC
CelA_S1_Forw	GGAG TATGGAAAAACTCAAGTTTATGCTT
CelA_S1_Rev	AAAC AAGCATAAACTTGAGTTTTTCCATA
Construction of pS_X^R_Δ*spoIIE*	
P1_Trna_F	TATTTTTTAAAAAAATTCAAGCTTGCATGC TCGCGGGGTGGAGCAGTTGGTAGC
16_0098_TRNA_R	TTTTGAAACTACTAC TTCCAAATAATGTAACTTGATTATAGCATC
16_TRNA_cbei_0098_F	TTACATTATTTGGAA GTAGTAGTTTCAAAAGTTTATTTAGATAGC
P2_0098_R	TACGAATTCGAGCTCGGTACCCGGGGATCC ATAAACCAGCGGATAATTTTTCAAGG
spoIIE_S1_Forw	GGAG ATTAATACTAATTACTTCTTTTCTT
spoIIE_S1_Rev	AAAC AAGAAAAGAAGTAATTAGTATTAAT
Construction of pSpoIIE	
Cbei0097prom_F	GACGTTGTAAAACGACGGCCAGTGCCAAGC ACGAATTTTGGTCACTTAAGCCAAG
Cbei0097_R	AGTCGACGTCACGCGTCCATGGAGATCTCG CTTATTGCATGATAATCTTAGCTATC
Construction of pCas9$_{ind}$-ΔcatB	
ΔcatB_fwd	TGTTATGGATTATAAGCGGCTCGAG GACGTCAAACCATGTTAATCATTGC
ΔcatB_rev	AATCTATCACTGATAGGGACTCGAG CAATTTCACCAAAGAATTCGCTAGC
Construction of pCas9$_{ind}$-ΔpNF2	
pNF2-gRNA	GAGCTCACTCTATCATTGATAGAGTTTGAAACTCTATCATTGATAGAGTATAATATCTTTGTTCATTTAAGCCATCTACTAAACAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAAGCTTGAGCTC
Primer used for screening	
Cbei_325_F (*celA*)	ATAAGTAACAATTAGATAATTATGAAGTTAATCCTTAG
Cbei_326_R (*celA*)	CATTTGCTTTCAGGTCTTCTTTTGCTG
Cbei_0096_F (*spoIIE*)	CGAAGATATTATGTCTAAGTTTCTA
Cbei_0098_R (spoIIE)	CATTACATTCCATACAATATTTATTGTATAAACCAGC
RH076 (*catB*)	CATATAATAAAAGGAAACCTCTTGATCG
RH077 (*catB*)	ATTGCCAGCCTAACACTTGG
pNF2_fwd (pNF2)	GGGCGCACTTATACACCACC
pNF2_rev (pNF2)	TGCTACGCACCCCCTAAAGG

Bold: restriction sites (**BsaI**); Underlined: non-specific tail;

Supplementary file S1: list of primers used in this study



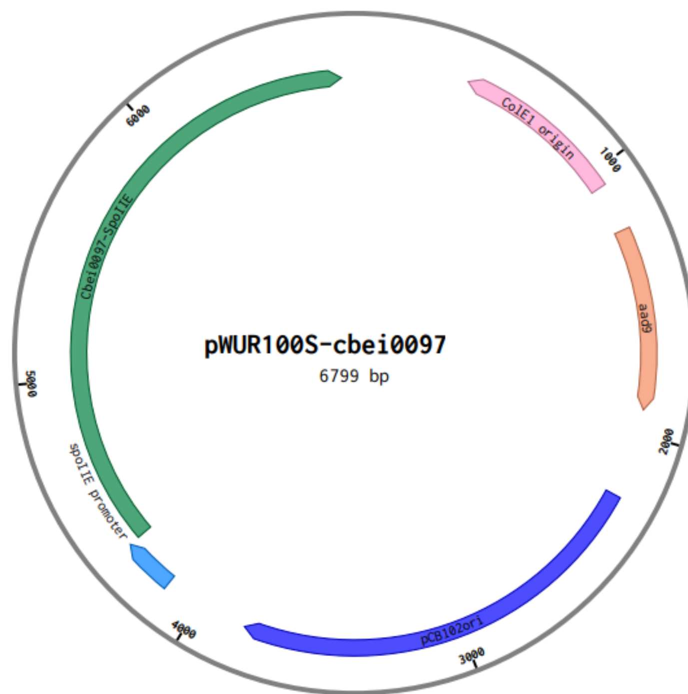
Supplementary file S2 : Maps of the plasmids pE_X_*cas9* (A) and pS_X^{R}KR (B). pAMβ1 replication origin; xylBp: promoter of the operon xylAB from *C. difficile*; $cas9_{opt}$: codon optimized *cas9* gene; colE1, gram negative replicon; *ampR*: gene resistance ampicillin; *ermB*: gene resistance erythromycin; 2µ: yeast replicon 2µ; *xylR*: gene of the regulation protein of the pxylB; *kan^{R}*: kanamycin resistance gene; Chimera: minimum sequence for gRNA (26); thl^{T}: terminator of the gene *thl* (cbei_3630). MCS: multi-cloning site; *aad9*: spectinomycin resistance gene, pCB102: replication origin.



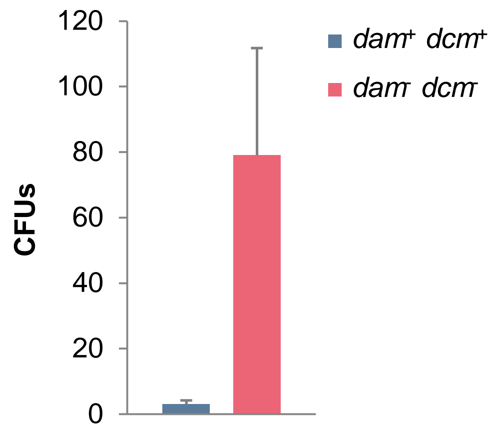
Supplementary file S3 : Allelic exchange events between the genomic DNA and editing DNA template. In our two plasmid system, the plasmid 2 harbours the gRNA directed against the target on the genomic DNA, the regulator of the promoter *xylB* from *C. difficile* sp. 630 and the editing DNA template consisting in the 2 sequences flanking the target in the genome (HS1 and HS2, Homologous Sequences) and a gene of interest (G.I.) inserted between the two homologous sequences. SAE: Single Allelic Exchange and DAE Double Allelic Exchange. In cells with a SAE, the target site is still present in the genome whereas is disappeared in the DAE cells. Strain harbouring SAE will then be sensitive to the activated CRISPR-Cas9 system

	concentration (g.L^{-1})					
	Acetate	Butyrate	Acetone	Butanol	Ethanol	Total ABE
wild-type	0.4 ± 0.0	0.1 ± 0.0	2.0 ± 0.5	7.7 ± 0.6	n.d	9.7 ± 0.6
∆spoIIE	0.4 ± 0.0	0.8 ± 0.0	2.7 ± 0.3	7.0 ± 0.1	n.d	9.7 ± 0.3

Supplementary file S2: Final products concentration in g.L-1 after a 72 h-fermentation experiments in 100 mL bottles of C. beijerinckii NCIMB 8052 wild type and ∆spoIIE strain. Values are given ± SEM of biological duplicates. Fermentations were performed in CM2 medium with 50 g glucose.



Supplementary file S6: Maps of the pSpoIIE plasmids: *colE1*, Gram negative replicon, *aad9*: spectinomycin resistance gene, pCB102: Gram positive replication origin.



Supplementary file S7: Comparison of transformation efficiencies in *C. beijerinckii* DSM 6423. 20 µg of the pCas9$_{ind}$ was used with and without *E. coli* Dam and Dcm methylations in triplicate experiments. Error bars represent the standard error of the mean (s.e.m.) of these replicates.

	concentration (g.L^{-1})				
	ethanol	acetone	isopropanol	butanol	total
wild-type	0.1 ± 0.0	0.2 ± 0.0	4.1 ± 0.1	7.0 ± 0.1	11.5 ± 0.1
ΔcatB	0.1 ± 0.0	0.1 ± 0.0	3.9 ± 0.1	6.8 ± 0.2	10.9 ± 0.3
ΔcatB ΔpNF2	0.1 ± 0.1	0.1 ± 0.0	4.5 ± 0.1	7.8 ± 0.5	12.5 ± 0.5

Supplementary file S8 : Final solvent concentration of 72h-fermentation experiments of *C. beijerinckii* DSM 6423 and derivative strains. Values are given ± SEM of biological triplicates. Fermentations were performed in Gapes medium (per litre: 2.5 g yeast extract, 1 g KH2PO4, 0.6 g K2HPO4, 1 g MgSO4 7H2O, 6.6 mg FeSO4 7H2O, 100 mg p-aminobenzoic acid, 2.9 g ammonium acetate, 60 g glucose).