

## SUPPORTING INFORMATION

### Toolkit development for cyanogenic and gold biorecovery chassis

*Chromobacterium violaceum*

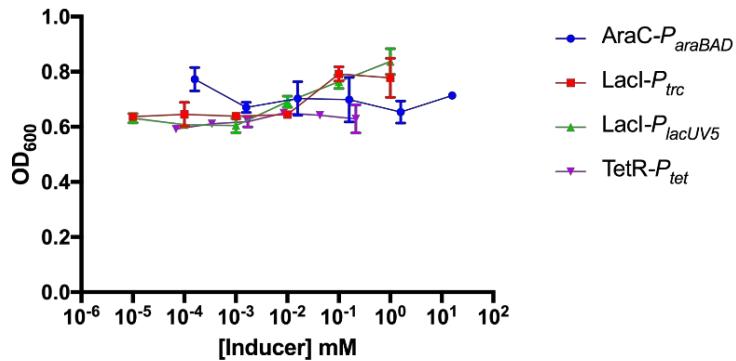
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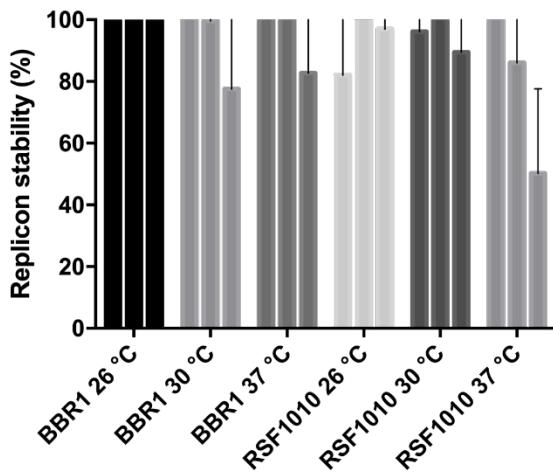
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## Supplementary figures



**Figure S1.** End-point optical density of cultures upon induction of different concentration of inducers. (0.00016 mM to 16 mM arabinose for AraC-P<sub>araBAD</sub>, 0.00001 mM to 1 mM IPTG for LacI-P<sub>lacUV5</sub> and LacI-P<sub>trc</sub>, 0.000068 mM to 0.214 mM aTc for TetR-P<sub>tet</sub>). Each value is the mean of biological duplicates, and error bars indicate  $\pm$  standard deviation.



**Figure S2.** Replicon stability of the BBR1 and RSF1010 origin vector through three cell passages at 26 °C, 30 °C and 37°C in *C. violaceum*. Each set of three bars represent the first, second and third cell passage respectively.

**Table S1. Broad host range plasmids used in this study.**

Plasmid name	Information	Reference
pBBR1-kan-pBAD-RFP (pltl1-64)	Vector with BBR1 origin and kanamycin resistance gene	This work
pBBR1-chlor-pBAD-RFP (pltl3-34)	Vector with BBR1 origin and chloramphenicol resistance gene	This work
pBBR1-tet-pBAD-RFP (pltl3-23)	Vector with BBR1 origin and tetracycline resistance gene	This work
pRSF1010-kan-pBAD-RFP (pltl3-48)	Vector with IncQ origin RSF1010 and kanamycin resistance gene	This work
pRSF1010-chlor-pBAD-RFP (pltl2-48)	Vector with IncQ origin RSF1010 and chloramphenicol resistance gene	This work
pSa-kan-pBAD-RFP (pltl4-60)	Vector with IncW origin Sa and kanamycin resistance gene	This work
pRK2-kan-pBAD-RFP (pltl2-74)	Vector with IncP origin RK2 and kanamycin resistance gene	This work

**Table S2. Broad host range plasmids used for replicon stability study.**

Plasmid name	Information	Reference
pBBR1-kan-pBAD-RFP (pltl1-64)	Vector with BBR1 origin and kanamycin resistance gene	This work
pRSF1010-kan-pBAD-RFP (pltl3-48)	Vector with IncQ origin RSF1010 and kanamycin resistance gene	This work

**Table S3. BBR1 plasmids used in this study for inducible promoters.**

Plasmid name	Information	Reference
pBBR1-kan-pBAD-RFP (pltl1-64)	Vector with BBR1 origin, kanamycin resistance gene and AraC-paraBAD inducible promoter	This work
pBBR1-chlor-pTrc-RFP (pltl3-24)	Vector with BBR1 origin, kanamycin resistance gene and LacI-pTrc inducible promoter	This work
pBBR1-tet-placUV5-RFP (pltl3-32)	Vector with BBR1 origin, kanamycin resistance gene and LacI-pLacUV5 inducible promoter	This work
pBBR1-kan-ptet-RFP (pltl3-36)	Vector with BBR1 origin, kanamycin resistance gene and TetR-pTet inducible promoter	This work

**Table S4.** BBR1 plasmids used for dCas studies.

Plasmid name	Information	Reference
pBBR1-kan-pBAD-dCas-J23119-random1gRNA (pltl5-67)	Vector with BBR1 origin, kanamycin resistance gene, AraC-paraBAD inducible promoter driving expression of dCas, strong constitutive promoter driving a non-targeting gRNA	This work
pBBR1-kan-pBAD-dCas-J23119-random2gRNA (pltl5-68)	Vector with BBR1 origin, kanamycin resistance gene, AraC-paraBAD inducible promoter driving expression of dCas, strong constitutive promoter driving a second non-targeting gRNA	This work
pBBR1-kan-pBAD-dCas-J23119-PvioAgRNA (pltl5-62)	Vector with BBR1 origin, kanamycin resistance gene, AraC-paraBAD inducible promoter driving expression of dCas and strong constitutive promoter driving a <i>vioA</i> promoter targeting gRNA	This work
pBBR1-kan-pBAD-dCas-J23119-vioBgRNA (pltl6-20)	Vector with BBR1 origin, kanamycin resistance gene, AraC-paraBAD inducible promoter driving expression of dCas and strong constitutive promoter driving a 5' UTR of <i>vioB</i> targeting gRNA	This work
pBBR1-kan-pBAD-dCas-J23119-vioCgRNA (pltl6-22)	Vector with BBR1 origin, kanamycin resistance gene, AraC-paraBAD inducible promoter driving expression of dCas and strong constitutive promoter driving a 5' UTR of <i>vioC</i> targeting gRNA	This work

**Table S5.** Broad host range origin sequences used in this study.





**Table S6. Antibiotic resistance sequences used in this study.**

**Table S7. Inducible promoter sequences used in this study.**

Part name	Sequence	Source
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	cactttatgcttcggcgtataatgtgtgaaattgtgagcggataacaa	
TetR-pTet	TtaagaccacttcacatTTAAGTTGTTCTAAATCGCATATGATCAATTCAAGGCCAATAAGAAGGCTG gctcgacaccttggtgatcaaataattcgatagcttgcgtataatggcgccactatcagttaggttt cccttcttctttagcacttgcgtatgccttgcgtatTTTCAACGCAACCTAAAGTAAAATGCCACAGCGCTGAG tgcataataatgcattctctatgtaaaaacccTTGGCATAAAAAGGCTAATTGATTTCGAGAGTTCTACT gttttctgtggccgtgtacctaattgtactttgcctatcgcatgacttagtaaaggcacatctaaaacttt agcgttattacgtaaaaatctgccagttcccttcaaggccaaatgtgatgtgtgcctatctaaca tctcaatggctaaggcgctcgagcaagcccccttatttttacatgccaataatgttaggtgtctacaccta gcttcggccgagttacgggtgttaaacctcgatccgacccatTAAGCAGCTCTAATGCGCTGTTAACAC tttacttttatctaattctagacatcatTAATTGTGACACTCTATCGTTGATAGAGTTATTTACAC tccctatcgatgatagagaa	pBbE2k-RFP (Addgene plasmid #35324) <sup>4</sup>

**Table S8. dCas and sgRNA sequences.**

	ggtgaaaaaggaaatcgagaagttaaaatcgtaaagagtactaggatcacaattatggaaagaagtcccttggaaa aaaatcgattgacttttagaagctaaaggatataagaagtaaaaaagacttaatcattaaactacctaataatagtctttt gagttagaaaaccggtcgtaaacggatgtggctagtgcggagaattacaaaaggaaatgagctggcttcggcaagcaaataat gtgaattttatatttagctgtcattatgaaaagttgaagggtagtcagaagataacgaacaaaacaattgttgtggagca gcataagcattathtagatgagattttagcattatcgtaatttctaaagcgtttagcagatgccaaatttagataaaagt tcttagtgcataacaaacatagagacaaaccataactgtgaacaagcagaaaaatatttcatatttgcgttgcacgatcttg gagctcccgctgtttaaatatttgatacaacattgtcgtaaacgcataactgttgcacgatcttgacatcttat ccatcaatccatcaactgtttatgaaacacgcattgttgcagtaggagggtgactga
Non-targeting sgRNA1	GATTCGTGCTTCGTTGAGAAgttttagagctagaaatagcaagttaaataaggctagtcgttatcaacttgaaa aagtggcaccgagtccggcgtc
Non-targeting sgRNA2	GACATAAGCCCTACGCCCATgttttagagctagaaatagcaagttaaataaggctagtcgttatcaacttgaaa aagtggcaccgagtccggcgtc
PvioA sgRNA	CGTCCGCCGTTGCCGCGCGGgttttagagctagaaatagcaagttaaataaggctagtcgttatcaacttgaaa aaagtggcaccgagtccggcgtc
vioB sgRNA	CGTCGCCGCCGGTTCCGGGgttttagagctagaaatagcaagttaaataaggctagtcgttatcaacttgaaa aaagtggcaccgagtccggcgtc
vioC sgRNA	AGAGCAATCATAGTCGGAGGgttttagagctagaaatagcaagttaaataaggctagtcgttatcaacttgaaa aaagtggcaccgagtccggcgtc

## REFERENCES

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