Recursive DNA assembly using Protected Oligonucleotide Duplex Assisted Cloning (PODAC)

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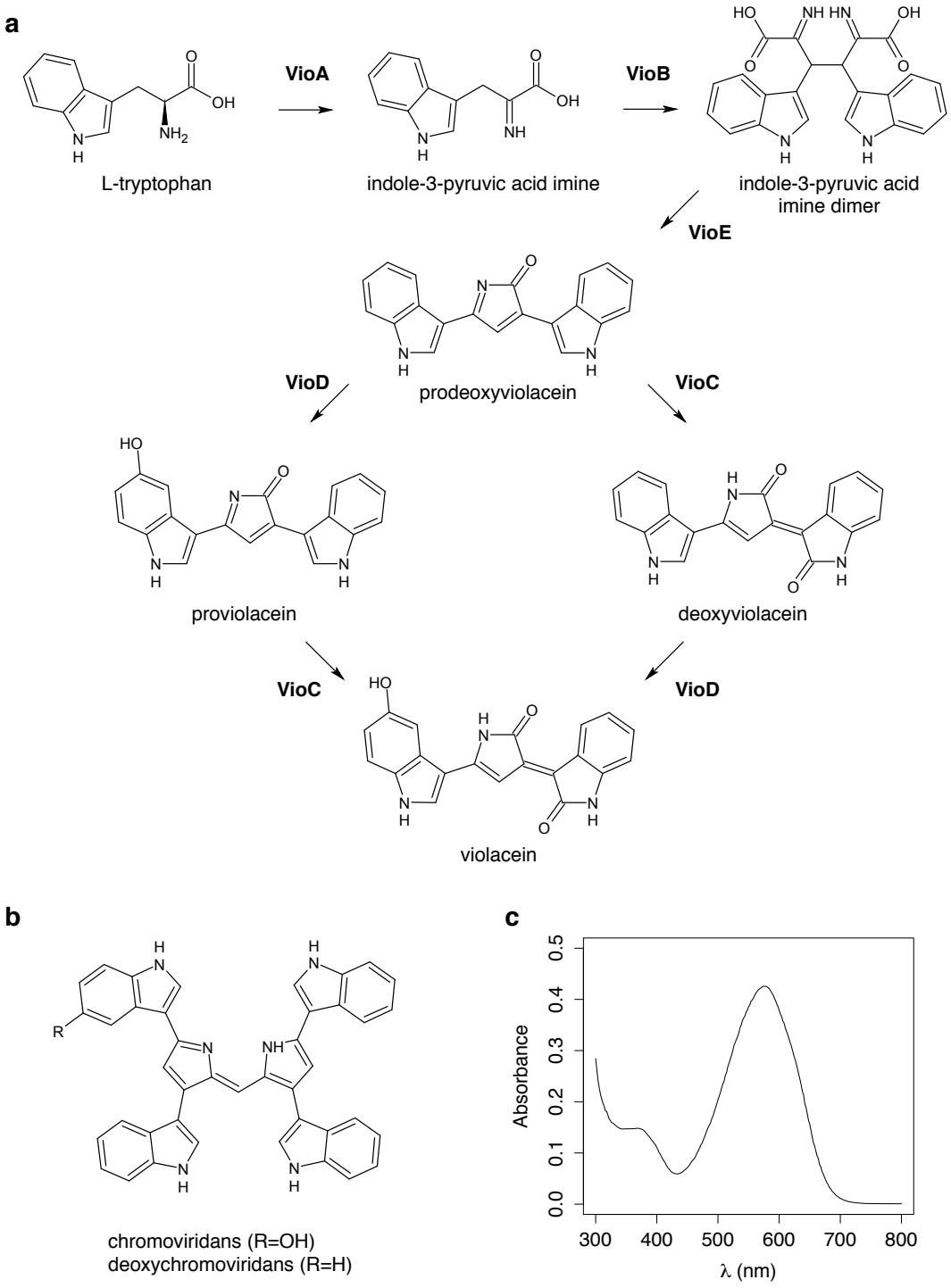
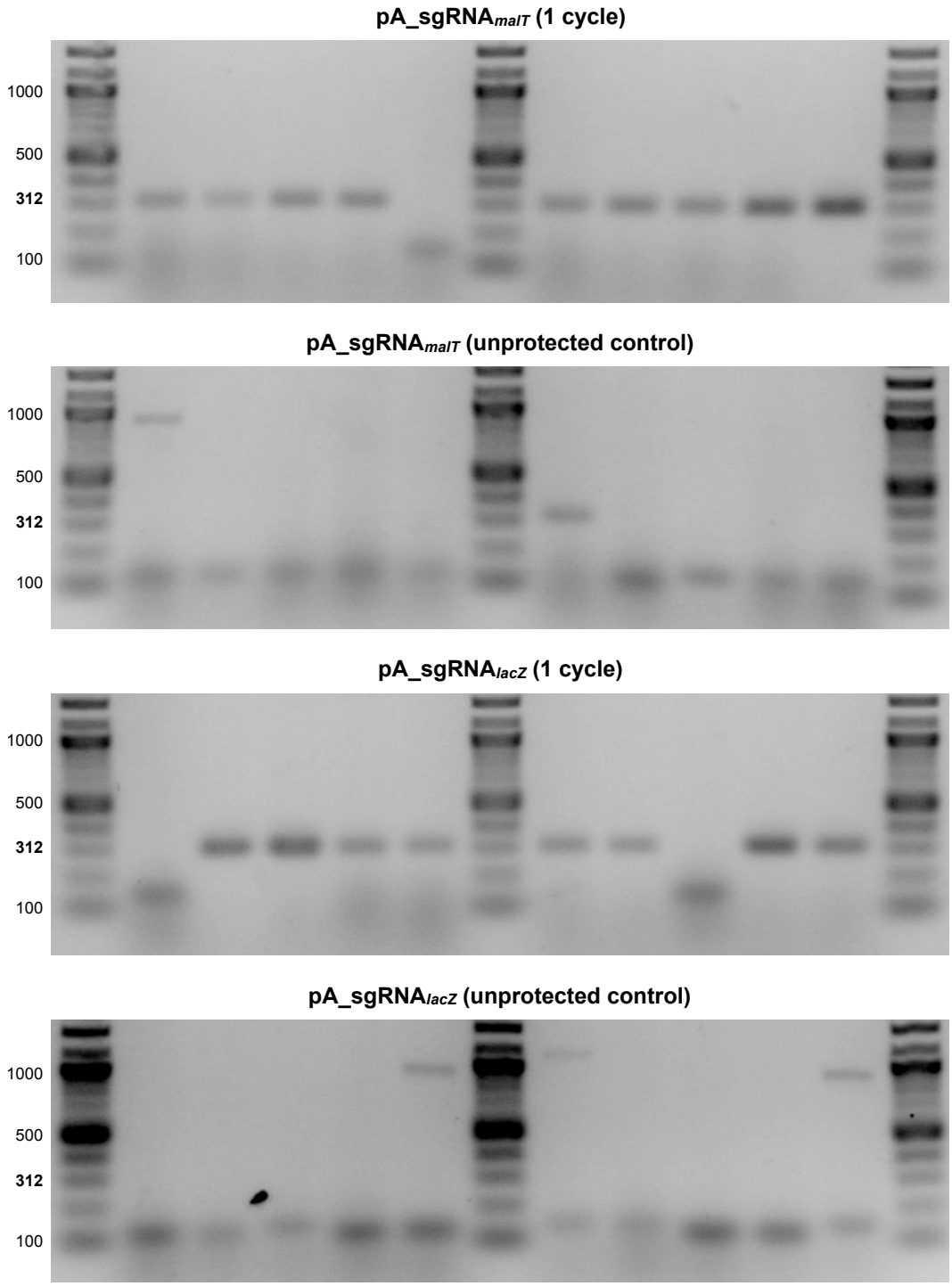
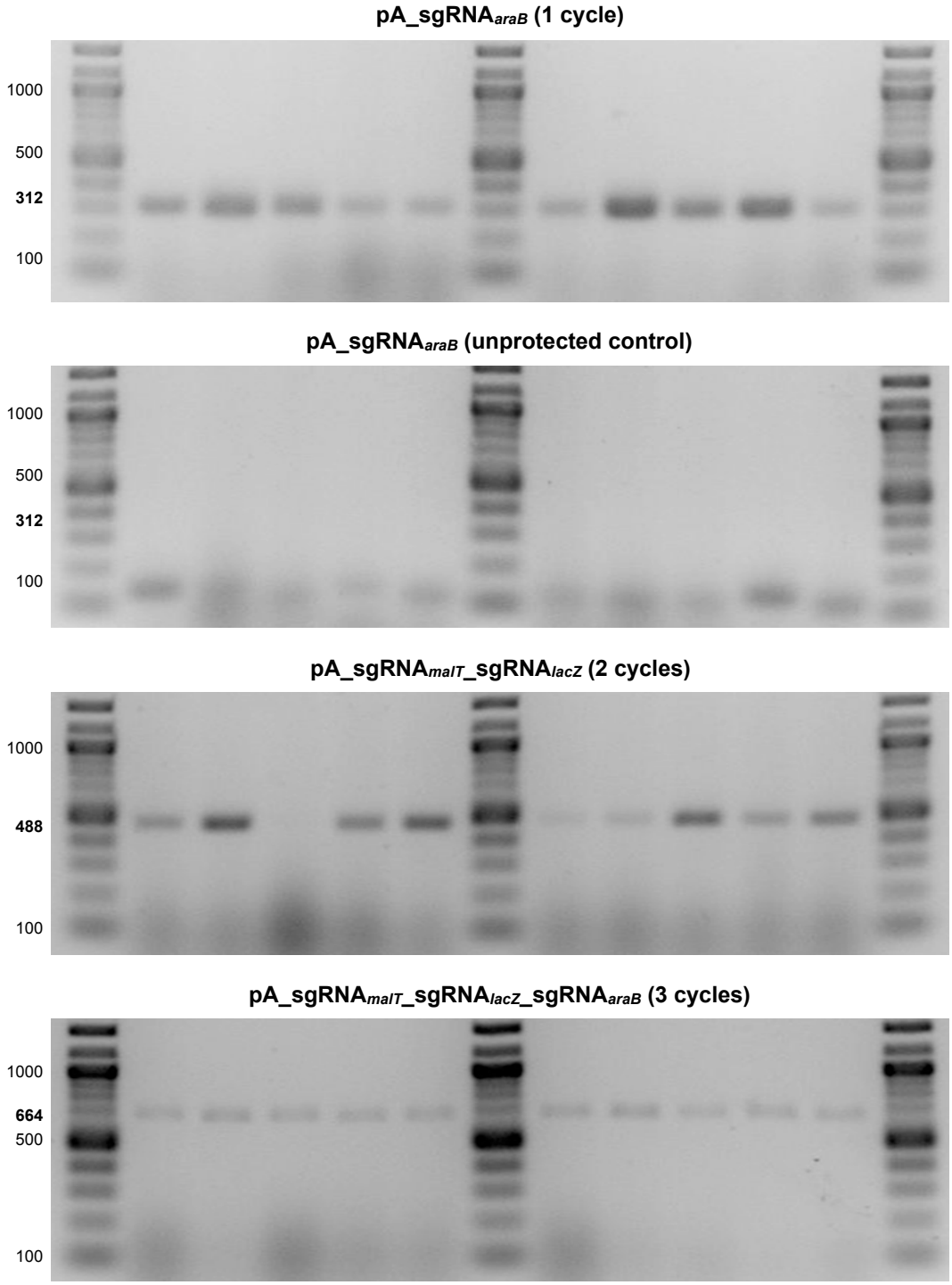
Supporting information



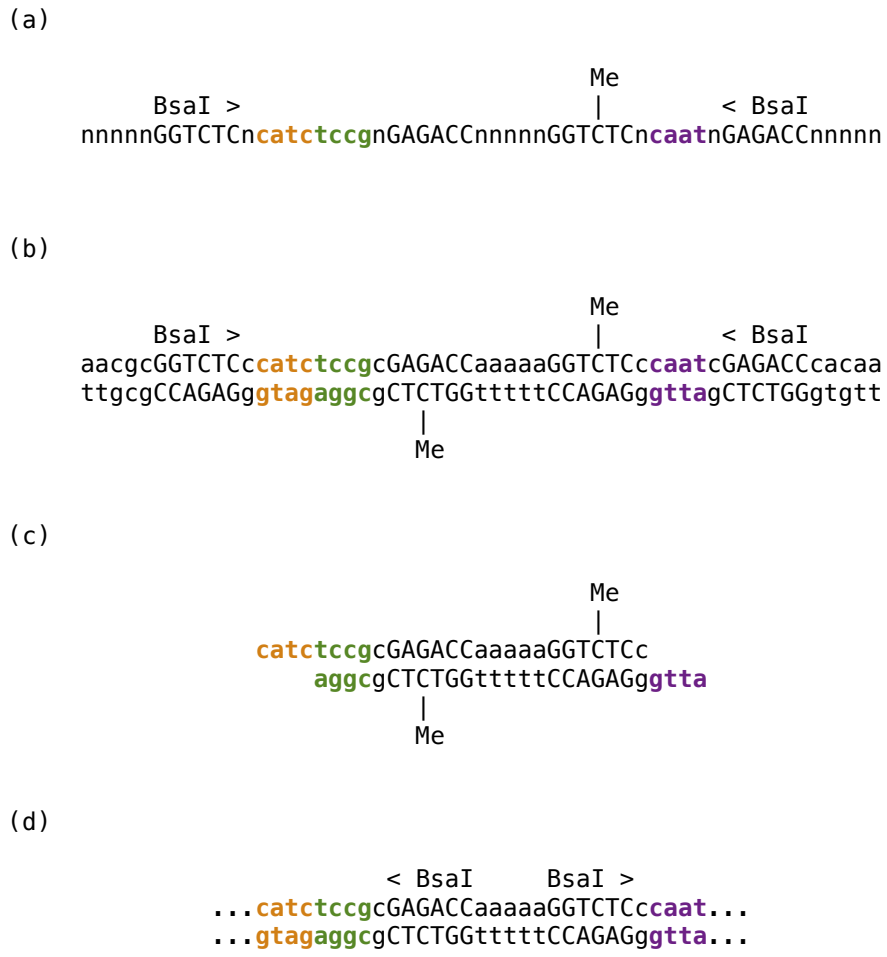
Supplementary figure S1: **Biosynthesis and properties of violacein and intermediates** (A) Deoxyviolacein and violacein are purple and dark blue colored compounds; (B) proviolacein and prodeoxyviolacein accumulation is visualized by the formation of the green condensation products chromoviridans and deoxychromoviridans, respectively. (C) Absorbance spectrum of culture pellet of *E. coli* carrying the *vioABEDC* operon, extracted with ethanol.



Supplementary figure S2: **PODAC sgRNA array assembly.** Results of colony PCR after PODAC reactions. Controls were performed under identical conditions but with an unprotected oligonucleotide duplex. Expected band length shown in bold.

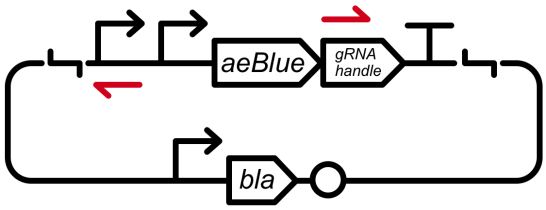


Supplementary figure S2: *Continued.*



Supplementary figure S3: **Oligonucleotide duplex design.** Sequence of PODAC oligonucleotides in the design phase (a) as well as before (b), during (c), and after (d) the assembly reaction.

(1)



(2)

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                    5' GTCCTAGGTATAATACTAGTxxxxxxxxxxxxxxxxxxxxGTTTTAGAGCTAGAAATAGC 3'

...CTAAAGATCTTTGACAGCTAGCTCA                                    GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG...
...GATTTCTAGAAACTGTCGATCGAGT                                    CAAAATCTCGATCTTTATCGTTCAATTTTATTCC...

    3' CTAGAAACTGTCGATCGAGTCAGGATCCATATTATGATCA 5'
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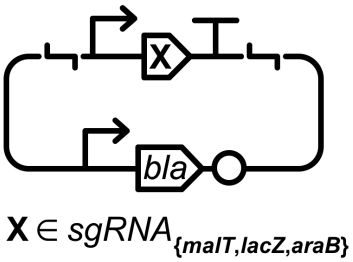
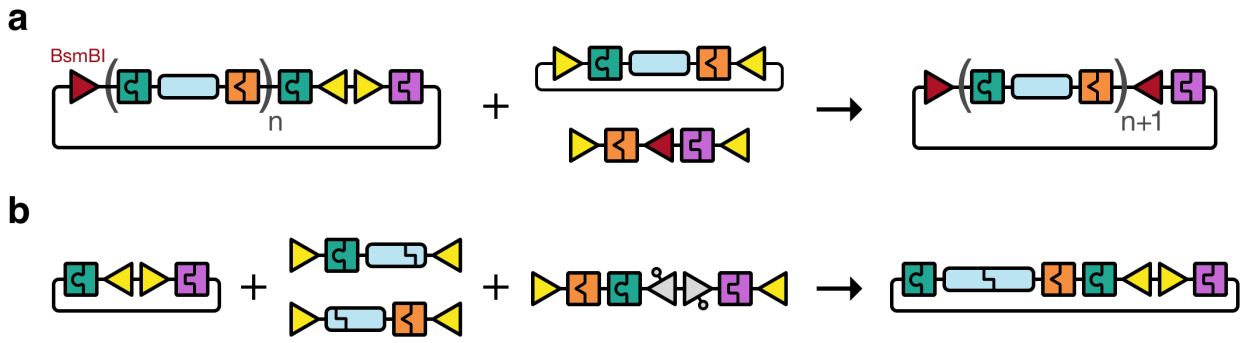
(3)



Supplementary figure S4: **SSA strategy used to construct pD-sgRNA plasmids.** Single Strand Assembly (SSA) was used as a fast and cost-effective method for the creation of individual sgRNA constructs. (1) A linear backbone is amplified from pD-sgRNA_blue using PCR; (2) CPEC is performed using a fixed anti-sense oligonucleotide and a variable sense oligonucleotide containing the CRISPR protospacer sequence; (3) The resulting construct encodes a functional sgRNA. Colonies containing template background can be selected against visually due to the expression of a blue chromoprotein.



Supplementary figure S5: **PODAC system variants.** See Figure 1 for legend and mechanism information. (a) *Subcloning mode*: The sgRNA acceptor vector was designed to include a BsmBI recognition site upstream of the PODAC cloning site. After iterative array assembly, a second BsmBI site can be introduced using a different oligo duplex. This allows for subcloning of the assembled construct using the BsmBI enzyme, into any PODAC acceptor plasmid and without additional scars. (b) *Fast mode*: multiple PCR products are cloned at once, without having to construct donor plasmids first. Fast mode PODAC forgoes the benefit of standardization and sequence accuracy in favor of reduced assembly time.

Supplementary table S1: **Case study 1: PODAC assembly of metabolic pathways.** Colony counts and assembly accuracy, as determined by phenotype, for 5 different PODAC reactions. The corresponding genotypes have been verified separately using standard molecular techniques. Methylation of the oligonucleotide duplex resulted both in higher assembly efficiency and accuracy compared to controls that were performed with an unprotected duplex under identical conditions.

Reaction	**PODAC**	**Control**
vioAB+E	245×10^2 (98.8%)	35×10^2 (62.4%)
vioABE+C	$> 600 \times 10^3$ (>99%)	62×10^3 (98.4%)
vioABEC+D	$> 600 \times 10^3$ (>99%)	21×10^3 (76.2%)
vioABE+D	$> 600 \times 10^3$ (ND*)	45×10^3 (ND*)
vioABED+C	$> 600 \times 10^3$ (>99 %)	17×10^3 (92.2%)

**vioABE* and *vioABED* colonies are similar in appearance