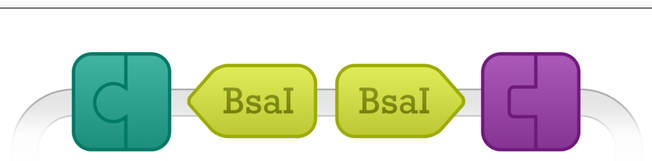
Constructing artificial CRISPR guide RNA arrays using methylated oligonucleotides

Bob Van Hove, Lien De Wannemaeker, Jo Maertens & Marjan De Mey
Metabolic Engineering Group - Laboratory of Industrial Biotechnology and Biocatalysis - Ghent University - Belgium

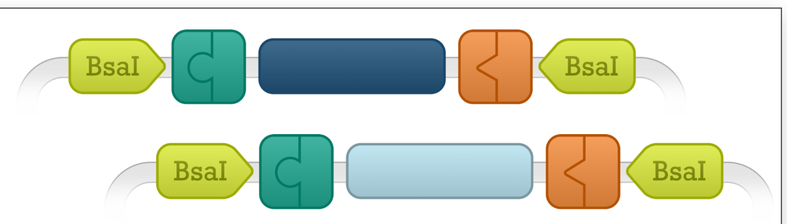
Introduction

The sequence repeats present in CRISPR arrays complicate the use of PCR and homology directed cloning methods. Modern restriction/ligation-based methods such as Golden Gate cloning do not suffer from this limitation[1]. However, because the resulting construct should be devoid of the restriction enzyme's recognition site, additional parts cannot easily be cloned into the same plasmid. As a solution, we present **PODAC: Protected Oligonucleotide Duplex Assisted Cloning**.



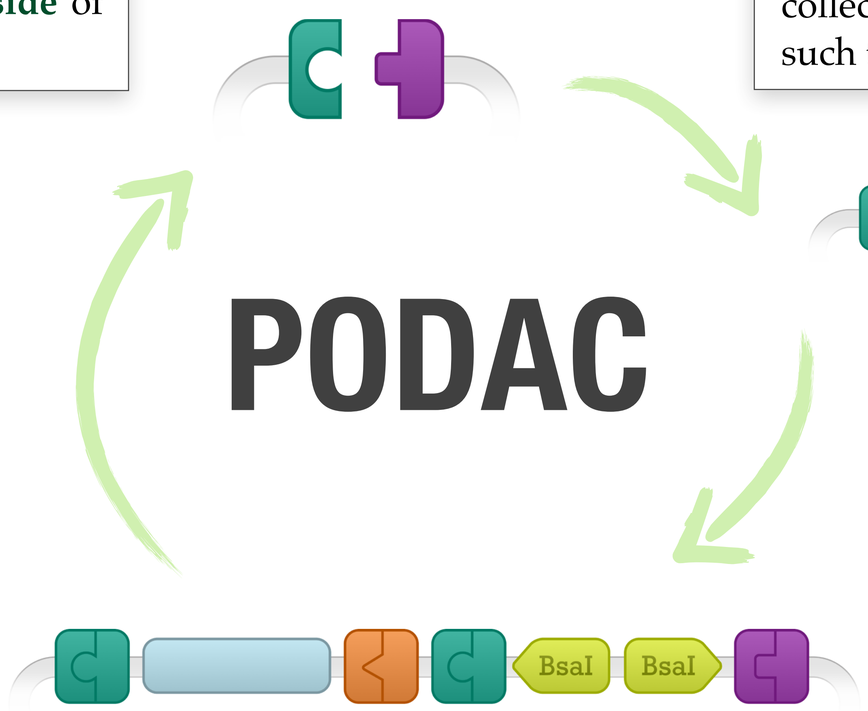
Acceptor

The Acceptor vector contains recognition sites for *BsaI*, a restriction enzyme that cleaves **outside** of its binding site.



Donor

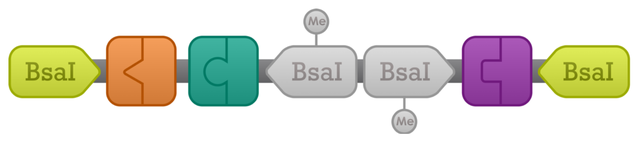
Genetic parts to be cloned are obtained from a collection of Donor plasmids containing *BsaI* sites such that complementary sticky ends are created.



Reactivation

Restriction and ligation are performed simultaneously and the product is transformed into *E. coli*. The main idea behind PODAC is that DNA replication in *E. coli* does not maintain the introduced methylation pattern.

E. coli **activates** the *BsaI* sites, resulting in a new Acceptor that can receive another part in the next iteration. Donor vectors and duplex are reusable because the sticky ends do not change.



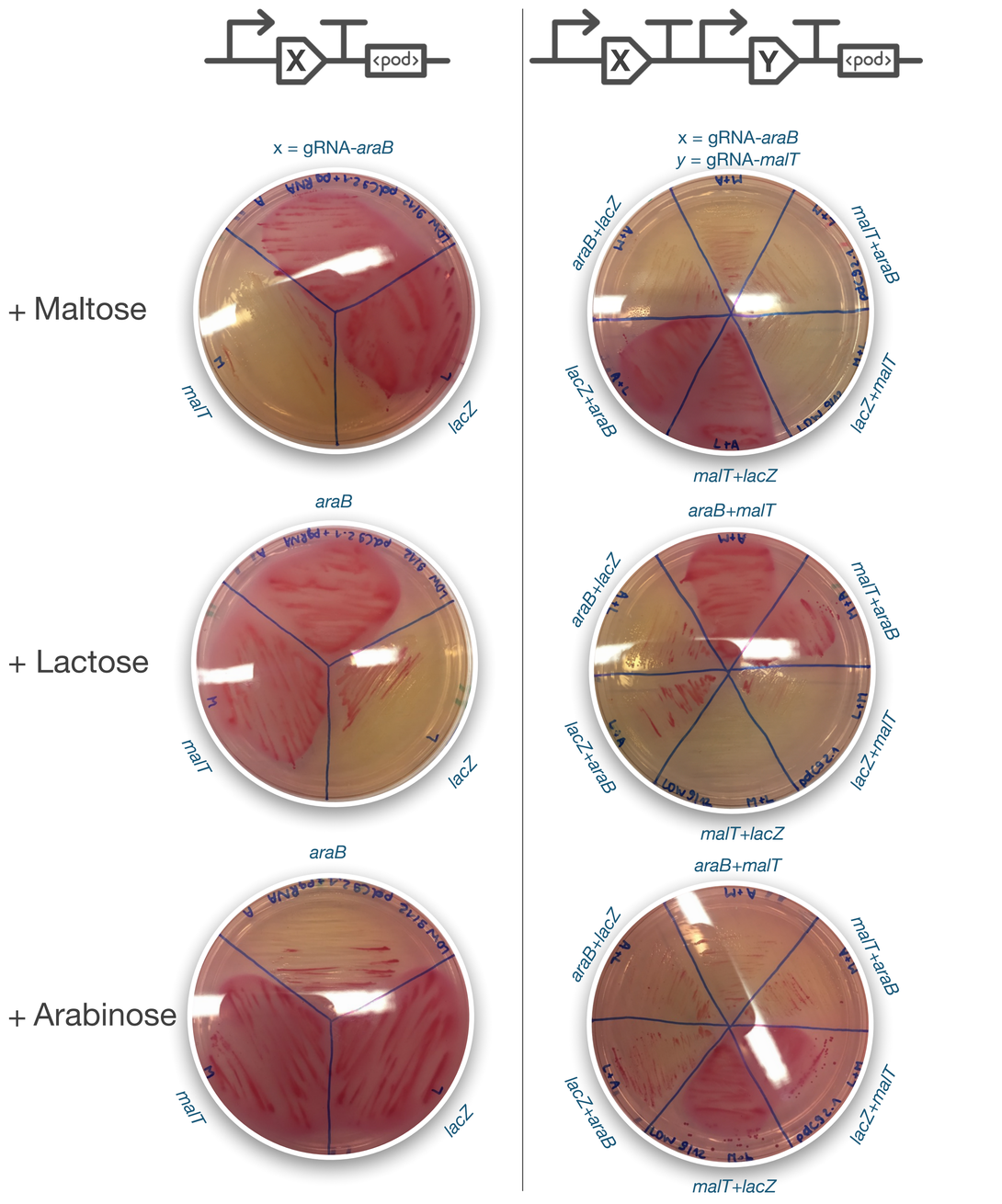
Protected duplex

PODAC introduces a new cloning site in the form of annealed oligonucleotides. The internal *BsaI* cloning site has been **deactivated** due to cytosine methylation, protecting them from digestion during the Golden Gate reaction[3].

CRISPR guide RNA arrays

We used PODAC to create artificial **CRISPR guide RNA arrays** for use in transcriptional roadblock mediated gene knockdown[4]. Each Donor plasmid delivers a single guide RNA with promoter and terminator. Unlike natural CRISPR regions, the resulting artificial arrays do not require processing into individual units[2].

Multiple genes related to the carbohydrate metabolism of *E. coli* were targeted, enabling a visual screen based on acidification of medium to which the specific sugar was added. The absence of red colour indicates a successful gene knockdown as these cells are unable to ferment the added carbohydrate.



References

(1) Engler, C., et al. (2008) A One Pot, One Step, Precision Cloning Method with High Throughput Capability. *PLoS ONE 3*, 3647–3654.
(2) Jinek, M., et al. (2012) A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science 337*, 816–821.
(3) Storch, M., et al. (2015) BASIC: A New Biopart Assembly Standard for Idempotent Cloning Provides Accurate, Single-Tier DNA Assembly for Synthetic Biology *4*, 781–787.
(4) Qi, L. S., et al. (2013) Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression. *Cell 152*, 1173–1183.

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bob.vanhove@ugent.be
bit.ly/atgtaa