CRISPR Golden Gate Assembly Design Automation

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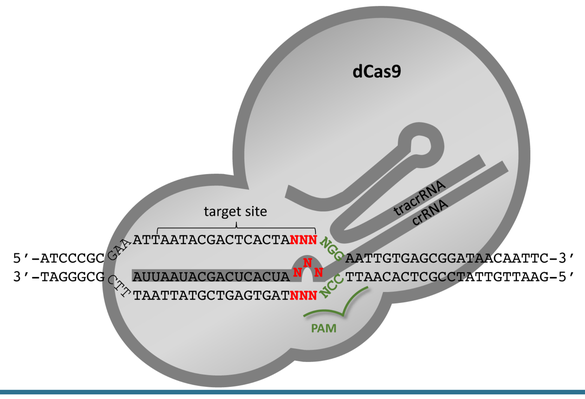
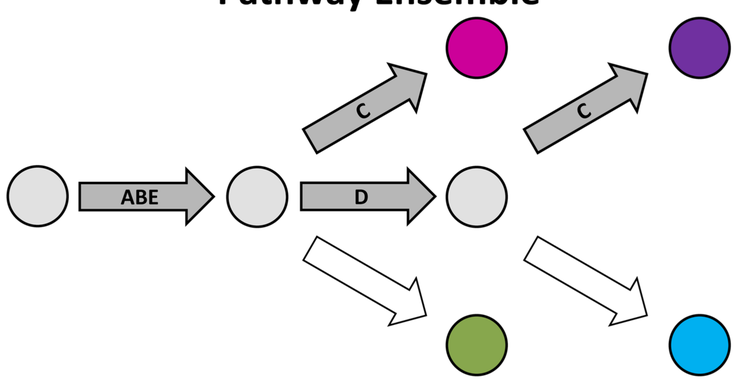
Background

A RNA-guided, DNA-binding protein known as dCas9 can be harnessed for metabolic engineering in *E. coli* by silencing genes that compete with the pathway of interest.

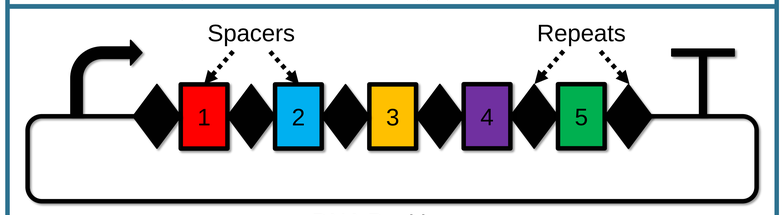


Ensemble of the violacein biosynthetic pathways and products. Major products include **prodeoxyviolacein**, **proviolacein**, **deoxyviolacein**, and **violacein**.

Pathway Ensemble



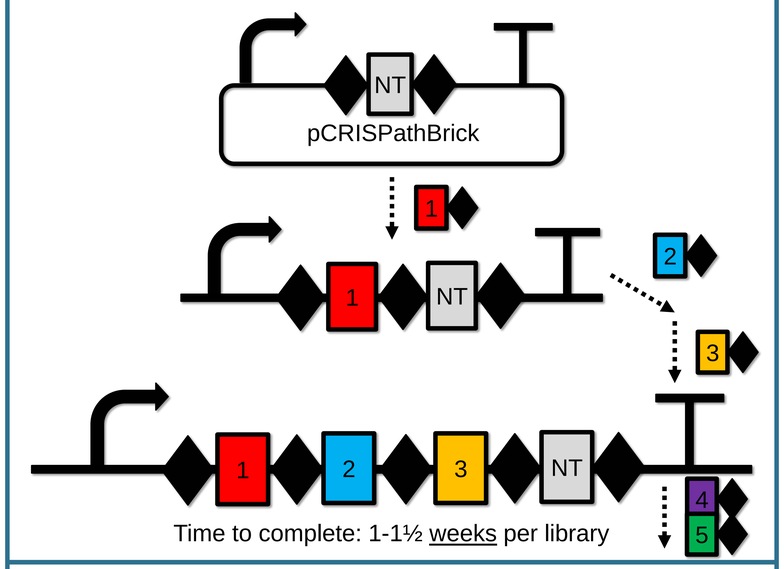
The instructions guiding dCas9 to appropriate binding locations are encoded in a DNA element known as a CRISPR array, composed of **spacers** which are regularly interspersed with short sequences known as **repeats**.



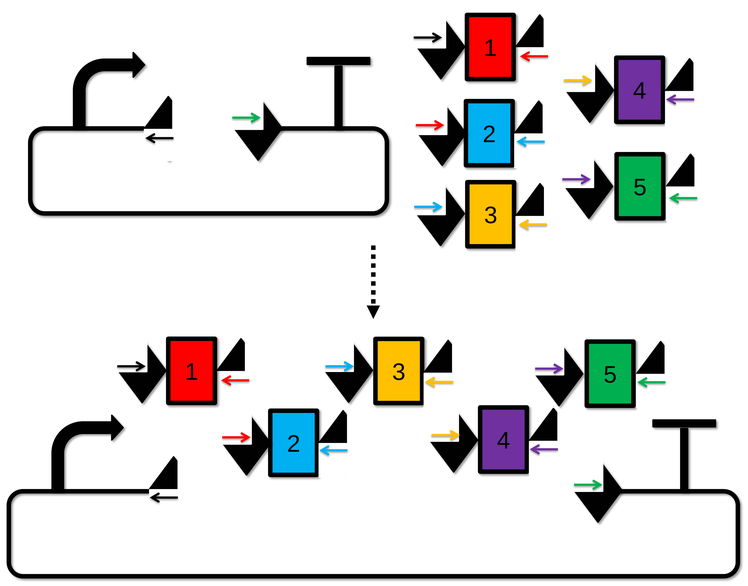
DNA Backbone

Constructing CRISPR Arrays

Construction of a CRISPR array has traditionally been a cumbersome task, while purchasing the arrays can be prohibitively expensive. Below is a typical array construction method, which requires a round of cloning for each spacer.



The Golden Gate assembly strategy allows researchers to construct libraries of CRISPR arrays in a one-pot reaction by the method shown below.



Automation and Implications

We have designed a MATLAB script which automates the selection of overhangs within the CRISPR repeat region. The algorithm has been successfully applied to construct Type II-A (Cas9) and Type V-A (Cpf1) CRISPR arrays.

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DNA Prior to any cutting:
TCTAATTTCTACGTTTGTAGA [D30] CTAATTTCTACGTTTGTAGAT
AGATTAAAGATGCAAACATCT [D30] GATTAAAGATGCAAACATCTA

DNA backbone after cutting out the dropout:
TCTAATTTCTACGTTTG           CTAATTTCTACGTTTGTAGAT
AGATTAAAGATGCAAACATCT           AAAGATGCAAACATCTA
-------------------------------------------------------------
Primer Orientation:
  5'-GGCCGAAGAC
TCTAATTTCTACGTTTGTAGAT   1   TCTAATTTCTACGTTTGTAGAT
AGATTAAAGATGCAAACATCTA       AGATTAAAGATGCAAACATCTA
                                          CAGAAGCCGG-5'
PCR Amplicon:
     GGCCGAAGACTGTAGAT   1   TCTAATTTCTACGTTTGGTCTTCGGCC
     CCGGCTTCTGACATCTA       AGATTAAAGATGCAAACCAGAAGCCGG
Digested Amplicon:
                 TAGAT   1   TCTAATTTCTA
                     A       AGATTAAAGATGCAA
-------------------------------------------------------------
Primer Orientation:
5'-GGCCGAAGAC
    TCTAATTTCTACGTTTGTAGAT   2   TCTAATTTCTACGTTTGTAGAT
    AGATTAAAGATGCAAACATCTA       AGATTAAAGATGCAAACATCTA
                                          CAGAAGCCGG-5'
PCR Amplicon:
   GGCCGAAGACTACGTTTGTAGAT   2   TCTAATTTCTACGGTCTTCGGCC
   CCGGCTTCTGATGCAAACATCTA       AGATTAAAGATGCCAGAAGCCGG
Digested Amplicon:
                CGTTTGTAGAT   2   TCTAATT
                    ACATCTA       AGATTAAAGAT
-------------------------------------------------------------
Primer Orientation:
5'-GGCCGAAGAC
        TCTAATTTCTACGTTTGTAGAT   3   TCTAATTTCTACGTTTGTAGAT
        AGATTAAAGATGCAAACATCTA       AGATTAAAGATGCAAACATCTA
                                          CAGAAGCCGG-5'
PCR Amplicon:
   GGCCGAAGACTTTCTACGTTTGTAGAT   3   TCTAATTGTCTTCGGCC
   CCGGCTTCTGAAAGATGCAAACATCTA       AGATTAACAGAAGCCGG
Digested Amplicon:
                TCTACGTTTGTAGAT   3   T
                    GCAAACATCTA       AGATT
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References

1. Cress, B.F., Toparlak, O.D., Guleria, S., Lebovich, M., Stieglitz, J.T., Englaender, J.A., Jones, J.A., Linhardt, R.J. and Koffas, M.A., 2015. CRISPathBrick: modular combinatorial assembly of type II-A CRISPR arrays for dCas9-mediated multiplex transcriptional repression in E. coli. *ACS synthetic biology*, *4*(9), pp.987-1000.
2. Cress, B.F., Jones, J.A., Kim, D.C., Leitz, Q.D., Englaender, J.A., Collins, S.M., Linhardt, R.J. and Koffas, M.A., 2016. Rapid generation of CRISPR/dCas9-regulated, orthogonally repressible hybrid T7-lac promoters for modular, tuneable control of metabolic pathway fluxes in Escherichia coli. *Nucleic acids research*, p.gkw231.