Stupar Lab Meeting
Multiplex CRISPR Design

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2017-06-19

Slides: https://z.umn.edu/crispr_slides

Publication in *The Plant Cell*

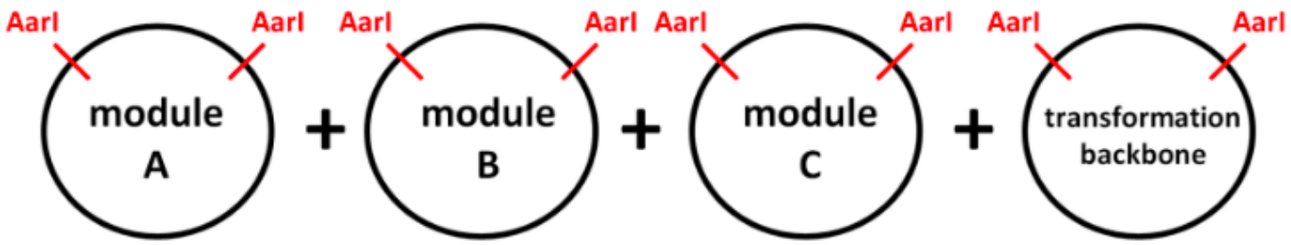
- Čermák et al. (2017). A Multi-purpose toolkit to enable advanced genome engineering in plants. *The Plant Cell*.
- DOI: **https://doi.org/10.1105/tpc.16.00922**

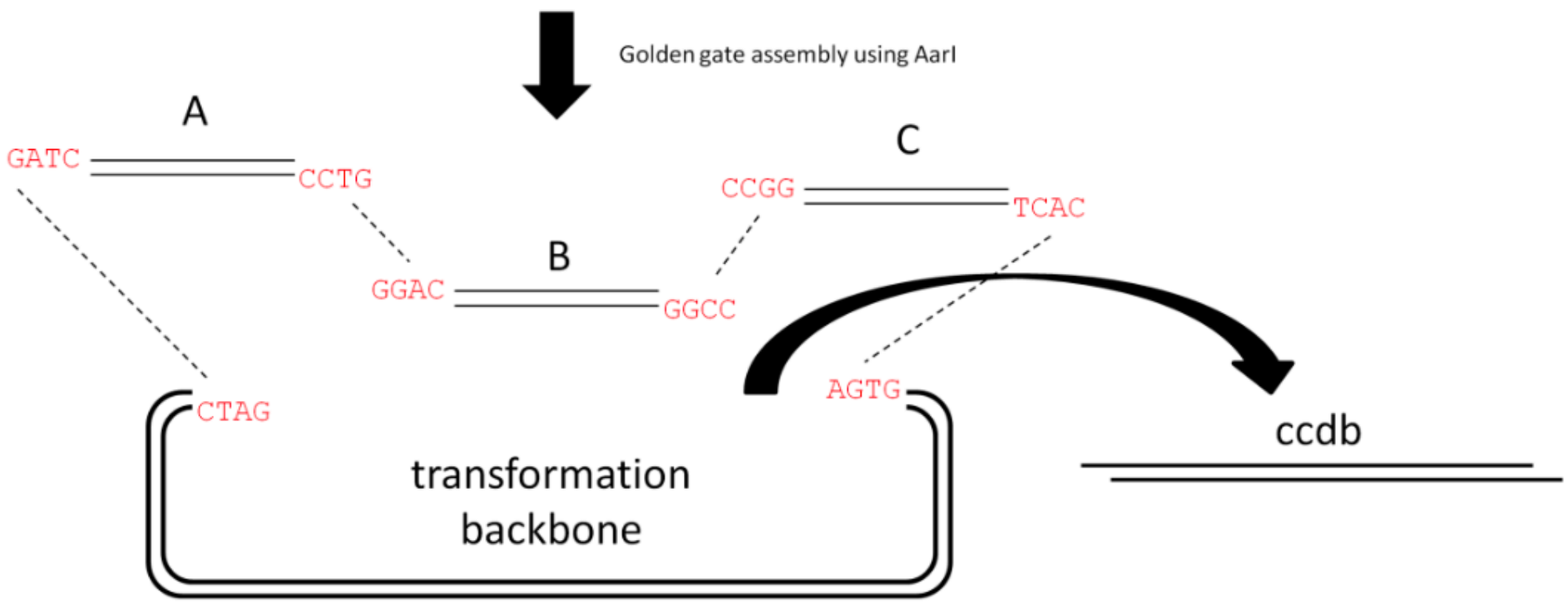
- See the paper for a description of the protocols, and the details of the methods

- Website: **http://z.umn.edu/crisprmultiplex**
  Note: Requires Javascript

Reagent Toolkit and Protocol

- A modular set of vectors that are built separately, then combined with a Golden Gate protocol





Reagent Toolkit and Protocol

Module	Number of Vectors	Description
Module A	61	Ready-to-use, with Cas9 or GFP cassettes
Module B	22	Add additional gRNAs or TALEN monomer
Module C	22	Add additional gRNAs, donor template, or expression cassettes
Transformation Backbone	31	Will be transformed into plant

Reagent Toolkit and Protocol

- Set of five protocols, with variants depending on what is being assembled (gRNAs, or TALENs)

Module B

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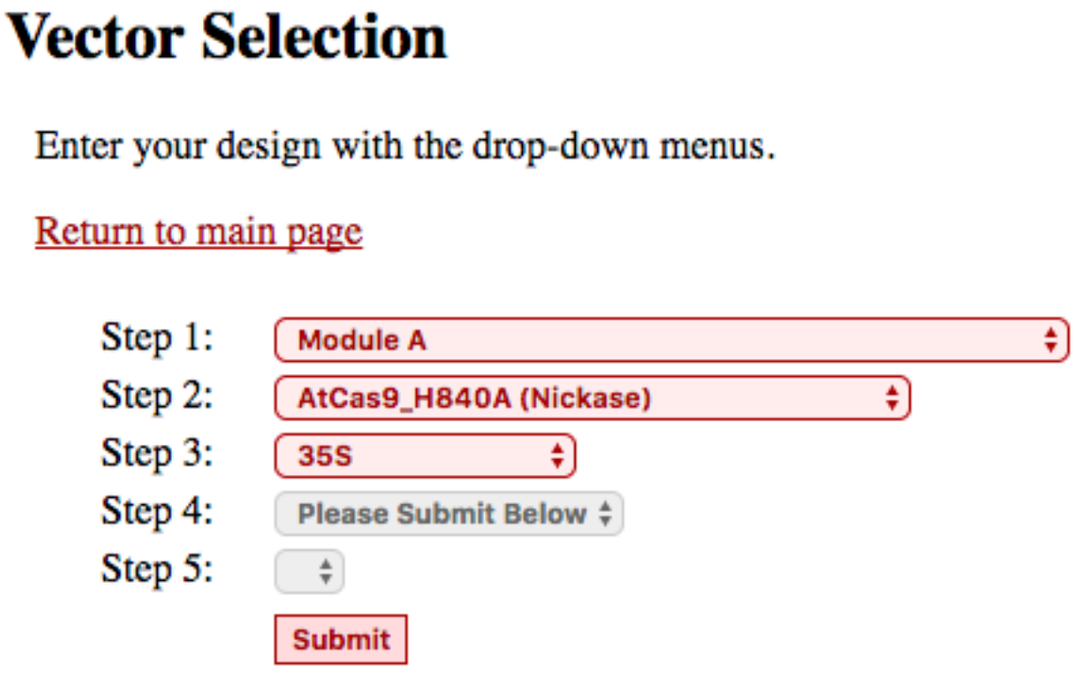
Plasmid ID	Gene	Promoter	Terminator	Protocols
pMOD_B0000	None	None	None	5
pMOD_B2000	TALEN_2 backbone with Esp3I ccdb cassette for repeat cloning	None (begins with P2A to be fused to TALEN 1)	HSP	1A, 5
pMOD_B2101	SapI ccdb cassette for cloning multiple gRNA protospacers with Csy4 spacers	35S	35S	3A, 3S2, 5
pMOD_B2103	SapI ccdb cassette for cloning multiple gRNA protospacers with Csy4 spacers	CmYLCV	35S	3A, 3S2, 5
pMOD_B2103b	SapI ccdb cassette (promoter in the backbone, not in the assembly) for cloning multiple gRNA protospacers with Csy4 spacers	CmYLCV	35S	3A, 3S1, 3S2, 5

Vector Selection

- Use drop-down menus to choose your vector
- Menus automatically update, and it will serve a vector map as a GenBank Flat File



Primer and Map Construction

- Accepts FASTA file of target sequences
- Select target vector, promoter, restriction enzyme, and splicing system

Multi-gRNA Array Assembly - Primer Design and Map Construction

Please enter the following design parameters into the form. Note that not every vector from the Vector Selection page is available in this construction tool.

Return to the main page

Targets (FASTA):	Choose File favorite targets.fasta
Target Vector:	pMOD_B2103
Promoter System:	35S
Restriction Enzyme:	BsaI
Splicing System:	Csy4
	Submit

Primer and Map Construction

Uploaded! Your file had 6 target sequences.

You are using the 35S promoter, the BsaI resctriction enzyme, and the Csy4 splicing system. You have chosen pMOD_B2103 as your target vector.

Primer Designs

PCR Reaction 1

```
>o35S
TGCTCTTCGCGCATGGAGTCAAAGATTCAA
>CSY_gRNA11
TGGTCTCCTGGATCTATCATCTGCCTATACGGCAGTGAAC
```

PCR Reaction 2

```
>REP_gRNA11
TGGTCTCATCCAGATGTTCCGTTTTAGAGCTAGAAATAGC
>CSY_gRNA13
TGGTCTCCGAAGAAGAAGAACTGCCTATACGGCAGTGAAC
```

PCR Reaction 3

```
>REP_gRNA13
TGGTCTCACTTCAGACACGAGTTTTAGAGCTAGAAATAGC
>CSY_gRNA16
TGGTCTCCATATAATGCACCCTGCCTATACGGCAGTGAAC
```

Download GenBank File

Vector Map

[Download]

```
LOCUS       pMOD_B2103_favorite_targets_fasta
DEFINITION  MODULE B with CmYLCV:SapI ccdb cassette for cl
            spacers - Csy4 .
ACCESSION   urn
VERSION     urn
KEYWORDS    .
SOURCE      .
  ORGANISM  .
            .
FEATURES             Location/Qualifiers
     rep_origin      1..857
                     /modified_by="User"
                     /label="High Copy Ori"
     promoter        878..1414
                     /note="35S Promoter"
     misc_feature    1422..1441
                     /note="Csy4"
     misc            1442..1461
                     /note="gRNA11"
     misc_feature    1462..1537
                     /note="gRNA Repeat"
     misc_feature    1538..1557
                     /note="Csy4"
     misc            1558..1577
                     /note="gRNA13"
     misc_feature    1578..1653
```