Guidelines for highly efficient construction of diatom episomes using Gibson Assembly

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Abstract

This protocol presents a Gibson Assembly design for highly efficient construction of diatom episomes. We regularly observe >90% efficiency (efficiency = % of screened bacterial colonies containing the desired construct) following the steps presented here.

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Guidelines

[a] This protocol has been tested with pPtPBR1 episome backbone in the following two contexts:

1. streamlining construction of a variety of expression cassettes with NR (nitrate reductase) promoter and terminator sequences

2. transferring an existing expression casette from one episome to another

pPtPBR1 - as well as other - diatom episomes can be obtained via addgene.

[b] We recommend using NEBuilder to design Gibson Assembly primers.

Before start

This is not a Gibson Assembly tutorial such as this here, but a brief overview of a Gibson Assembly design which significantly increases the likelihood of correct assemblies. We also assume familiarity

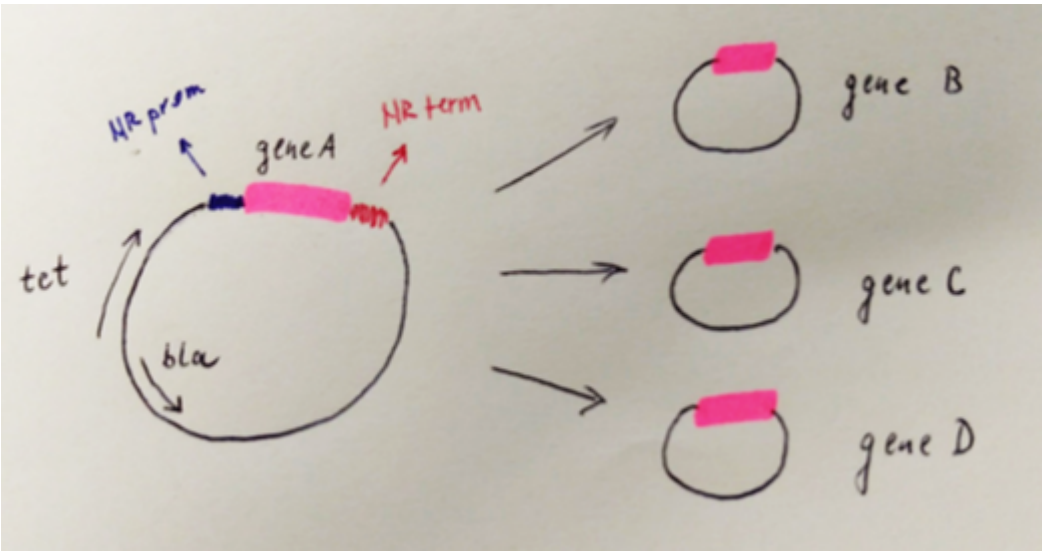
with diatom episome structure as reported in the literature.

Protocol

Case study: streamlining construction of protein expression cassettes

Step 1.

Here is an example of streamlining construction of protein expression cassettes using a promoter/terminator combo of your choice. See the example below using NR promoter/terminator:



tet: tetracycline resistance gene

bla: ampicillin resistance gene

Episome fragmentation & standardization

Step 2.

The crucial design principles employed here are:

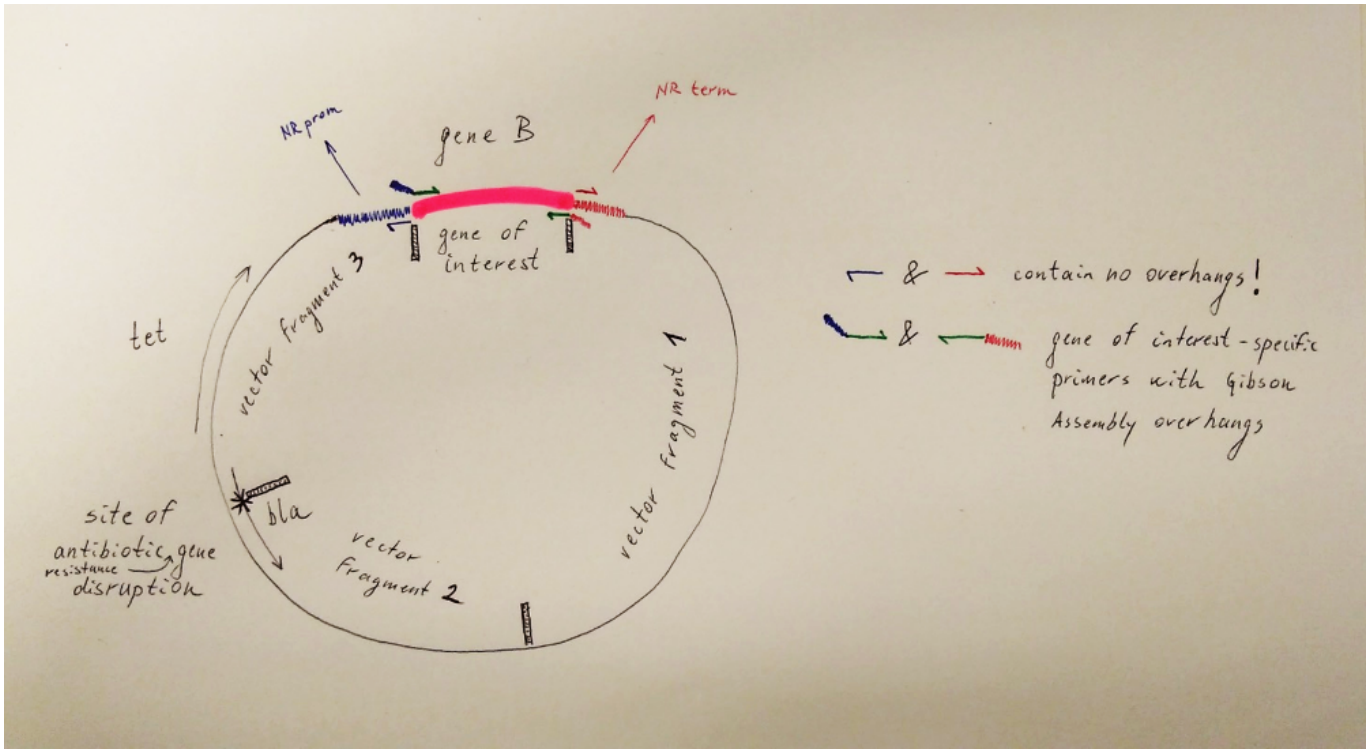
1. **disrupt antibiotic resistance gene and use that antibiotic for final selection** (fragmentation)

2. make sure that **Gibson Assembly overhangs are introduced via PCR of your gene of interest and not episome fragments** (standardization)

When we say 'standardization' we mean using the same set of episome-specific PCR fragments for all assemblies involving a certain promoter/terminator combo.

Diatom episomes are hard to PCR in full which is another reason for fragmentation. We note that promoter and terminator sequences can be separate fragments as well of course, but we also note that the overall Gibson Assembly efficiency will fall as a function of fragment number. Assembling up to 4 pieces simultaneously is very reliable though. If too many pieces preclude your assembly, we recommend sequential assembly.

See the generic episome fragmentation & strandardization scheme below:



Now in order to express any other gene of interest, one would only need to order one additional set of primers (changing only the green primer portion in the process).

Alleviating the need for DpnI treatment of your PCR fragments

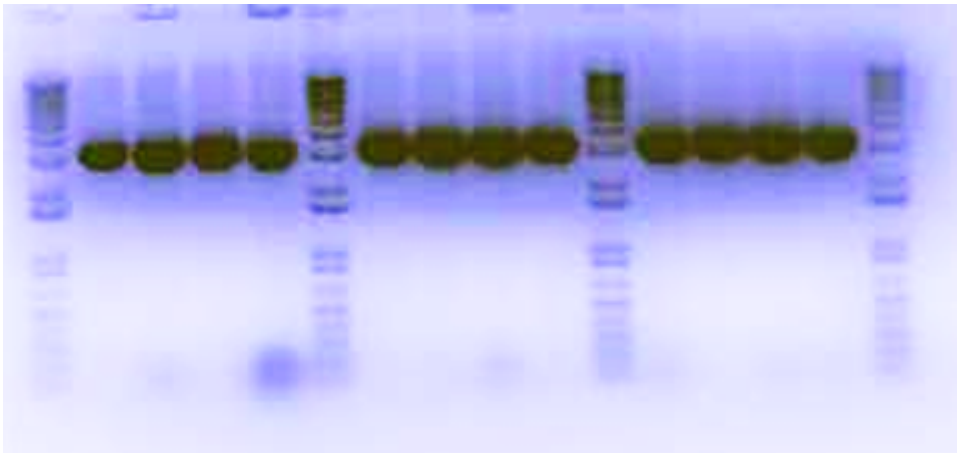
Step 3.

We recommend running the whole PCR reaction on a gel and **gel extracting the product**. This will (a) greatly minimize the risk of any template carry over which could lead to false positives and (b) allow you to proceed without additional treatment of your PCR product with DpnI. We note that the risk of template carry over is present only if your PCR template is a plasmid with either Amp or Tet resistance gene which are both present in diatom episome backbones (applies to the pPtPBR episome series).

Colony PCR screening

Step 4.

We routinely use OneTaq 2x Master Mix with Standard Buffer for colony PCR screening (we run 12.5 µL as opposed to 25 µL reactions listed as a minimum). Here is an example of a 4 piece Gibson Assembly where 12/12 tested colonies ended up being positive:



On distrupting antibiotic resistance genes

Step 5.

We recommend using either **fresh Amp100 LB plates** or preferably **carbenicillin** instead of ampicillin to minimize emergence of satellite colonies. Alternatively, we recommend **disrupting Tet resistance gene** where satelite colonies won't be a problem.

Warnings