General updating scheme for the design and construction of assembly vectors that are compatible with BBF RFC 10 and BBF RFC 28

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1 Purpose

BBF RFC 75 intends to link the BBF RFC 28-based combinatorial multi-part assembly method to BBF RFC 10. Its aim is to easily redesign an expression vector to make it compatible to BBF RFC 10 for the construction of fusion proteins based on BBF RFC 28. These vectors can then directly be used for expression of the fusion protein.

2 Relation to other BBC RFCs

BBF RFC 75 is an update on BBF RFC 28.

3 Copyright notice

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4 Description

BBF RFC 28 is a method for combinatorial multi-part assembly based on the Type II restriction enzyme AarI. It is a highly efficient method for the construction of fusion proteins. Unfortunately, it is not compatible with the widely applied and iGEM-required BBF RFC 10. As a consequence, the fusion proteins designed according to BBF RFC 28 are not combinable with BBF RFC 10-based vectors as they do not contain the required prefix and suffix sequences.

Here we give a general outline how one can easily convert an expression vector (that must contain a promoter region followed by at least two unique restriction sites) into a BBF RFC 28 and BBF RFC 10- compatible assembly vector. By assembly vector we mean a vector that can be used as acceptor vector for the fusion of three BBF RFC 28-compatible parts.

In general an assembly vector that can be used for the efficient assembly of multiple parts SHOULD contain an insert that is flanked by AarI-sites. Those cleavage sites MUST be designed such that AarI-digestion releases the insert and generates a vector backbone with 5' overhangs compatible with the BBF RFC 28 standard. The rationale behind cloning an insert into the vector instead of simply adding two AarI sites is that AarI does not cleave with the same efficiency on every recognition site. If the insert is ligated into the vector previously, the cleaved vector can be efficiently separated from uncut vector fractions by separation on an agarose gel. The insert can be any random sequence or code for e.g. a reporter gene.

As update on BBF RFC 28, we designed primers that can be readily used to amplify any insert of choice. These primers contain the prefix and suffix sequences that are required to make the vector consistent with BBF RFC 10, a ribosome binding site (RBS) for the forward

primer and AarI-sites which give BBF RFC 28 compatible overhang 9 bp from the RBS. These overhangs confirm to BBF RFC 28 when assembling three parts into the vector (vector plus three parts). AarI sites are designed to be located on the insert such that the vector will not contain AarI recognition sites anymore after release of the insert.

Further, one needs to add homologues regions for the insert of choice to the primer sequence and to add restriction sites that are suitable for subcloning into the vector of choice. When using a BBF RFC 10 compatible vector one does not need to add additional restriction sites.

Primers are designed as follows:

Forward:

```
5` yyy<u>GAATTC</u>GCGGCCGCT<u>TCTAGAGAGGAG</u>CCTGCGGAGTTTT<u>GCAGGTG</u>xxxx
Reverse:
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5` yyyyyCTGCAGCGGCCGCTACTAGTACGCAAAAAGCAGGTGxxxx
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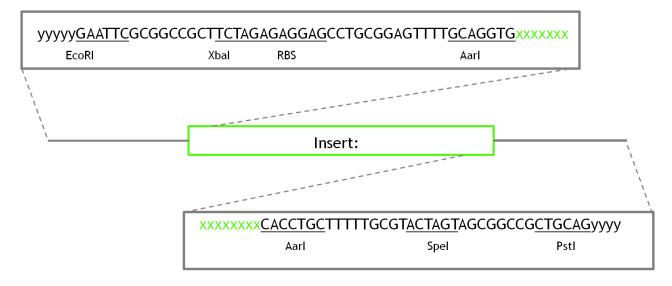
where xxxx is the region that MUST be homologues to the insert of choice and yyyy is any restriction site suitable for cloning into the vector of choice.

The general cloning procedure is outlined below:

1. Use the primers specified below to amplify your insert of choice. Primers encode for BBF RFC 10-specified suffix and prefix sequences and a ribosome binding site for direct protein expression which will be 9 bp from the start of the fusion protein.

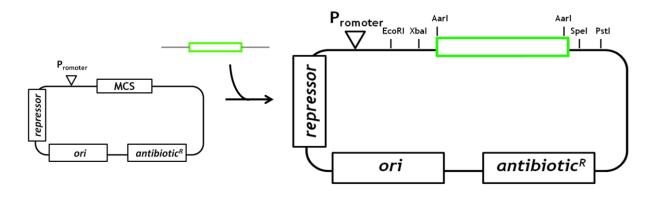
yyy = any restriction enzyme that is suitable for cloning into your vector of choice.

xxx= region homologues to your insert of choice.

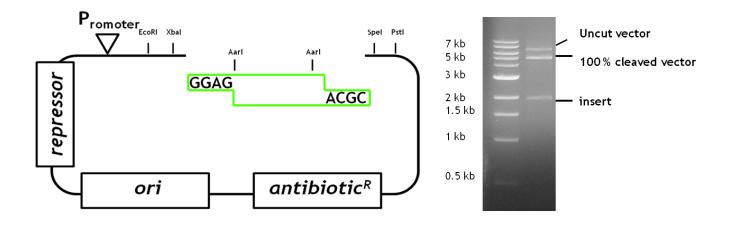


Primers: forward 5` yyyyyGAATTCGCGGCCGCTTCTAGAGAGGAGCCTGCGGAGTTTTGCAGGTGxxxxxx 3` reverse 5` yyyyyCTGCAGCGGCCGCTACTAGTACGCAAAAAGCAGGTG xxxxxxxxxxxx 3`

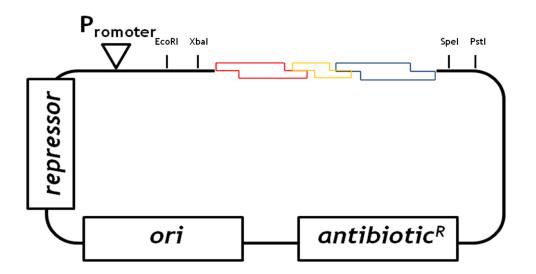
2. Clone the insert into your expression vector of choice to generate an assembly vector that is compatible with BBF RFC 10 and BB RFC 28.



3. Digest your assembly vector with AarI and separate the reaction mix on a 1% agarose gel to isolate 100% cleaved vector backbone.



- 4. Assemble your fusion protein of choice with the AarI-cleaved working vector....
- 5. ...and go for expression.



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