

BBF RFC 28: A method for combinatorial multi-part assembly based on the Type IIs restriction enzyme AarI

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1 Purpose

This BioBricks Foundation Request for Comments (BBF RFC) describes an alternative assembly standard based on the Type IIS restriction enzyme AarI.

2 Relation to other BBF RFCs

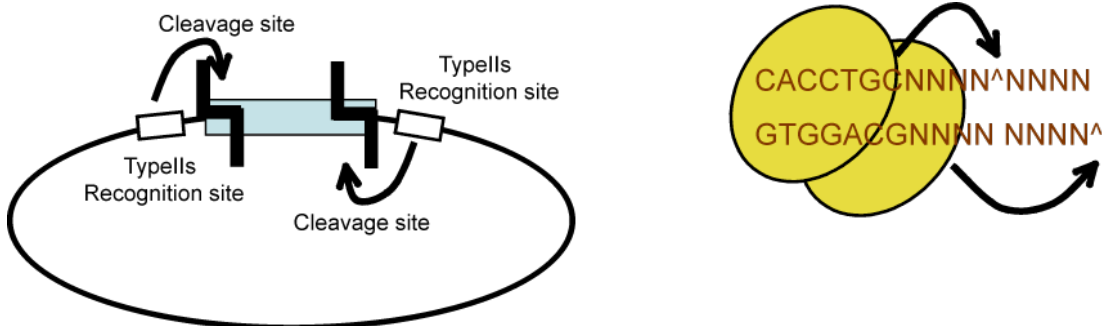
BBF RFC 28 does not update or replace any earlier BBF RFC.

3 Copyright Notice

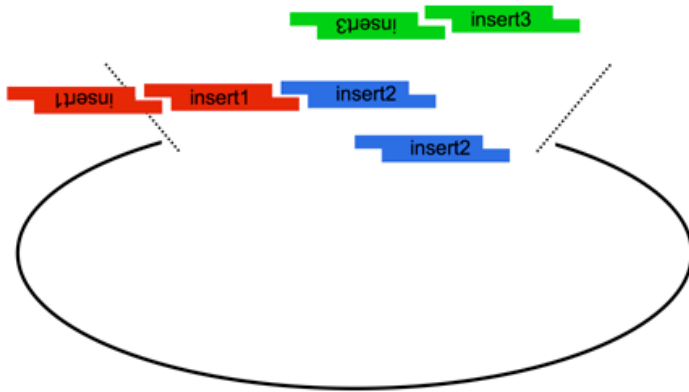
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4 Description

We are using a multi-part/combinatorial cloning technique that is particularly well suited to shuffling protein domains. The key to this approach is the Type IIS restriction enzyme, AarI, a rare (7-cutter) that cuts 4bp offset from its binding site. Thus, AarI can generate four base overhangs of any sequence.



Since the user can specify the overhangs, this method can be used to "stitch-together" fragments without a scar, which is sometimes necessary to preserve protein function. More importantly, these overhangs can be non-palindromic, which solves the biggest problem faced when trying to do multipart ligations using standard restriction enzymes, the self ligation of a part, blocking its incorporation into the construct. This problem is illustrated here:



By contrast, Aar1 cloning allows high efficiency ligations using up to 4 parts (vector plus 3 inserts). While parts can be made with any 4 base overhang (end), we chose a standard set, termed A, B, C, and D (underlined).

A-part-B: CACCTGC NNNN GGAG (nnn ... nnn n) CCCT NNNN GCAGGTG

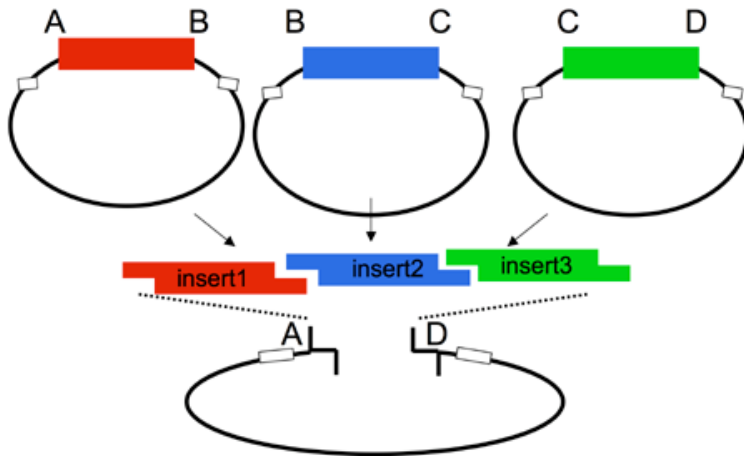
B-part-C: CACCTGC NNNN CCCT (n nnn ... nnn nn) GCGA NNNN GCAGGTG

C-part-D: CACCTGC NNNN GCGA (nnn ... nnn) TGCG NNNN GCAGGTG

B-part-D: CACCTGC NNNN CCCT (n nnn ... nnn nn) TGCG NNNN GCAGGTG

While care was taken to match frames between B and C ends, the presence of an ATG at the A end and the presence of a STOP at the D end can vary. Please check the respective part for further information.

This system allows parts to be traded between researchers. We are building a lab database of parts.



These ends yield 3 possible parts: AB, BC and CD. For two part ligations, we use AB and BD parts. Parts could be promoters, protein domains, or terminators, and are typically generated by PCR from a genomic DNA or plasmid template, then TOPO or Gateway BP cloned into **storage vectors** and sequenced. **Storage vectors**, once validated, can be shuffled with other validated parts, into **acceptor vectors**, creating large combinatorial libraries of constructs that do not require further sequencing.

While any vector can be adapted to be an acceptor for Aar1 cloning, we worked with the yeast pRS__ series of vectors in 2008 and have been using modified Gateway ENTR vectors in 2009. We have provided to the registry several types of acceptor vectors built in the pRS315 or 305 backbone, as well as acceptor vectors based on Gateway ENTR vectors.

5 Methods

For more information on how to clone with UCSF Aar1 parts, or better yet, to design your own Aar1 parts, we provide the following protocols:

How to design primers to generate your own Aar1 A-B, B-C or C-D parts:

- 1) Decide whether you want to generate an A-B, B-C or C-D part.
- 2) Pick the appropriate primer sequence from below.
- 3) Think about how you want to clone your part into a storage vector (TOPO TA, Gateway BP reaction, others?) and add the appropriate sequence to the 5' of your primer.
- 4) Add the sequence of the homology to your template at the 3' of your primer (where it says homology – written in the frame you want).

5) Think about whether you want your part to contain an ATG (START), a TAA (STOP), a Kozak sequence, etc and add appropriate sequence (examples given in red).

6) Double check the primer sequence and take special care of the frame!

A-B parts:

Primer A: 5' - CAC CTG CAA AAG GAG (ATG?) hom olo gy - 3'

Primer B: 5' - CAC CTG CAA AAA GGG A hom olo gy - 3'

B-C parts:

Primer B: 5' - CAC CTG CAA AAC CCT A hom olo gy - 3'

Primer C: 5' - CAC CTG CAA AAT CGC CT hom olo gy - 3'

C-D parts:

Primer C: 5' - CAC CTG CAA AAG CGA hom olo gy - 3'

Primer D: 5' - CAC CTG CAA AAC GCA (TTA?) hom olo gy - 3'

B-D parts:

Primer B: 5' - CAC CTG CAA AAC CCT A hom olo gy - 3'

Primer D: 5' - CAC CTG CAA AAC GCA CC (TTA?) hom olo gy - 3'

Color code:

yellow: the recognition sequence of Aar1 (that's where the enzyme sits)

green: 'buffer' bases

blue: site of cutting, those bases give the part its identity

Aar1 digestion protocol

How to combine parts from a storage vector into an acceptor vector.

Start with standard 5ml culture miniprep, eluted with 50ul. (should yield ~250 ng/ul)

FOR VECTORS and INSERTS:

Digest 20ul (~5 ug) of miniprep (use Aar1 specific buffer):

Water 30.6

Buffer	6
Oligo	0.9
DNA	20
Enzyme	2.5

3 hrs at 37 deg.

For VECTORS, PCR purify this reaction, and elute with 44ul.

PCR purification eluate

Buffer 4	5
BSA	0.5
Xma1	1.5

Digest 2 hrs at 37deg. Add 1ul CIP, incubate one more hour.

Gel Purify vectors and inserts. For weak bands, elute with 30ul, for strong use 50ul.

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7 Suggested Reading

The following papers describe a similar cloning strategy.

Golden gate shuffling: a one-pot DNA shuffling method based on type II restriction enzymes.

Engler C, Gruetzner R, Kandzia R, Marillonnet S.
PLoS One. 2009;4(5):e5553. Epub 2009 May 14.
PMID: 19436741

A one pot, one step, precision cloning method with high throughput capability.

Engler C, Kandzia R, Marillonnet S.
PLoS One. 2008;3(11):e3647. Epub 2008 Nov 5.
PMID: 18985154