BBF RFC 36: Fusion protein BioBrick assembly standard with optional linker extension

1   Purpose

This RFC 36 describes an extension of the original BioBrick assembly standard (BBF RFC10) and Freiburg assembly standard (BBF RFC 25). The Fusion Assembly strategy described here is fully compatible with RFC 25 (Freiburg) and RFC 10 standard and supports in-frame fusion and controlled potentially infinite extension of linker between protein fusion domains.

2   Relation to other BBF RFCs

RFC 36 extends RFC 25 and is transferrable to other RFC compatible with RFC 25.

3   Motivation

BioBrick Assembly standards are easy to use for cloning and some newly introduced standards also enable protein in-frame fusions. For precise structure building using protein or peptide domains a precise length of a linker is often required (e.g. for controlling the flexibility between protein domains), which may have to be optimized. Instead of having to prepare several versions of protein domains with extended linkers or a set of linkers, which are due to their small size difficult to clone the described standard allows extension of linker by 2 residues in each cloning cycle, which can be iterated at will. Residues in linkers are preferably small hydrophilic residues, since they are in most cases exposed to the solvent and they should contain residues that are either flexible (e.g. Gly, Ser, Thr) or conformationally constrained (e.g. Pro).
This RFC 36 is fully compatible to RFC 25 and also to RFC 10 and allows the construction of linker between two in-frame ligated coding sequences.

4   Description

Here we describe the properties of modified **BioBrick-NIC-II/-III** vector (BBF RFC 36 assembly strategy). All sequences defined herein are specified in the 5' to 3' direction.

Two newly introduced BioBrick assembly standards BioBrick-NIC-II (BB-NIC-II) and BioBrick-NIC-III (BB-NIC-III) allow protein in-frame cloning and enable iterative linker extension. They originate from standard BioBrick vector with altered cloning site.

The main reasons for modification of standard assembly strategy are:

(i) in-frame fusions of parts with friendly scars; and
(ii) to extend scar/linker between parts.

The proposed assembly strategy requires certain sequence properties for the part and the surrounding DNA.

4.1.1   BioBrick-NIC-II assembly description:

prefix: `5' gaattc(EcoRI) gcggccgc(NotI) ATG tctaga(XbaI) gccggc(NgoMIV) accggt(AgeI)`
suffix: `5' cccggg(XmaI) tccgga(BspEI) actagt(SpeI) TAA gcggccg(NotI) ctgcag(PstI)`

Two types of scars are formed with parts fusion and depend on used restrictions:

scar I using *Xba*I and *Spe*I: Thr-Arg
scar II using *Ngo*MIV and *Bsp*EI: Ser-Gly

With respect to restriction sites used for cloning parts into BB-NIC-II three different modifications to part could be achieved:

(i) no extension;
(ii) extension of part with Ser-Gly at the C-terminal site of insert; and
(iii) extension of part with Thr-Gly at the N-terminal site of insert.

4.1.2   BioBrick-NIC-III assembly description:

prefix: `5' gaattc(EcoRI) gcggccgc(NotI) ATG tctaga(XbaI) gccggc(NgoMIV) cccggg(XmaI)`
suffix: `5' accggt(AgeI) tccgga(BspEI) actagt(SpeI) TAA gcggccg(NotI) ctgcag(PstI)`

Fusion of two parts leaves two types of scar similar as above.

A standard scar Asp-Arg is formed using *Xba*I and *Spe*I restriction enzymes.
A friendly scar Ser-Gly is formed using *Ngo*MIV and *Bsp*EI restriction enzymes.

With respect to restriction sites using for cloning parts into BB-NIC-II four different modifications to part could be achieved:

(i) no extension;
(ii) part extension with Ser-Gly at C terminal site;
(iii) part extension with Pro-Gly at N terminal site; and
(iv) part extension with Pro-Gly at N terminal site and Ser-Gly at C terminal site.

4.2   Cloning

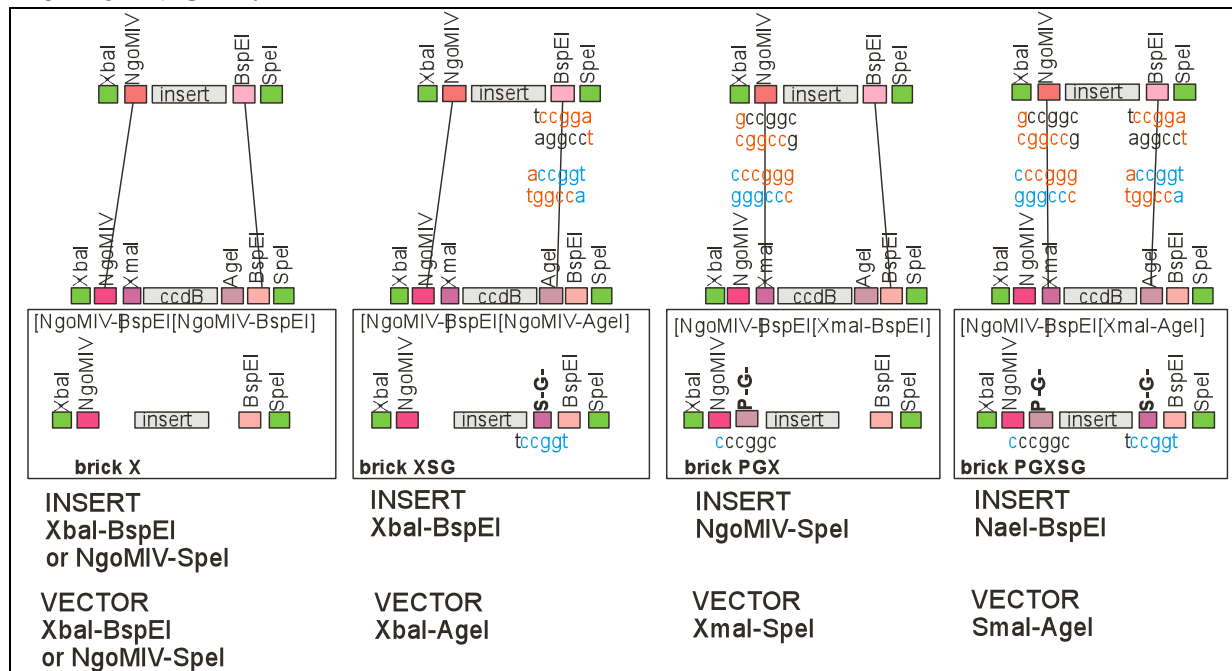Parts could be cloned differently into multiple-cloning site of **BB-NIC** vectors:

BioBrick-NIC-II:
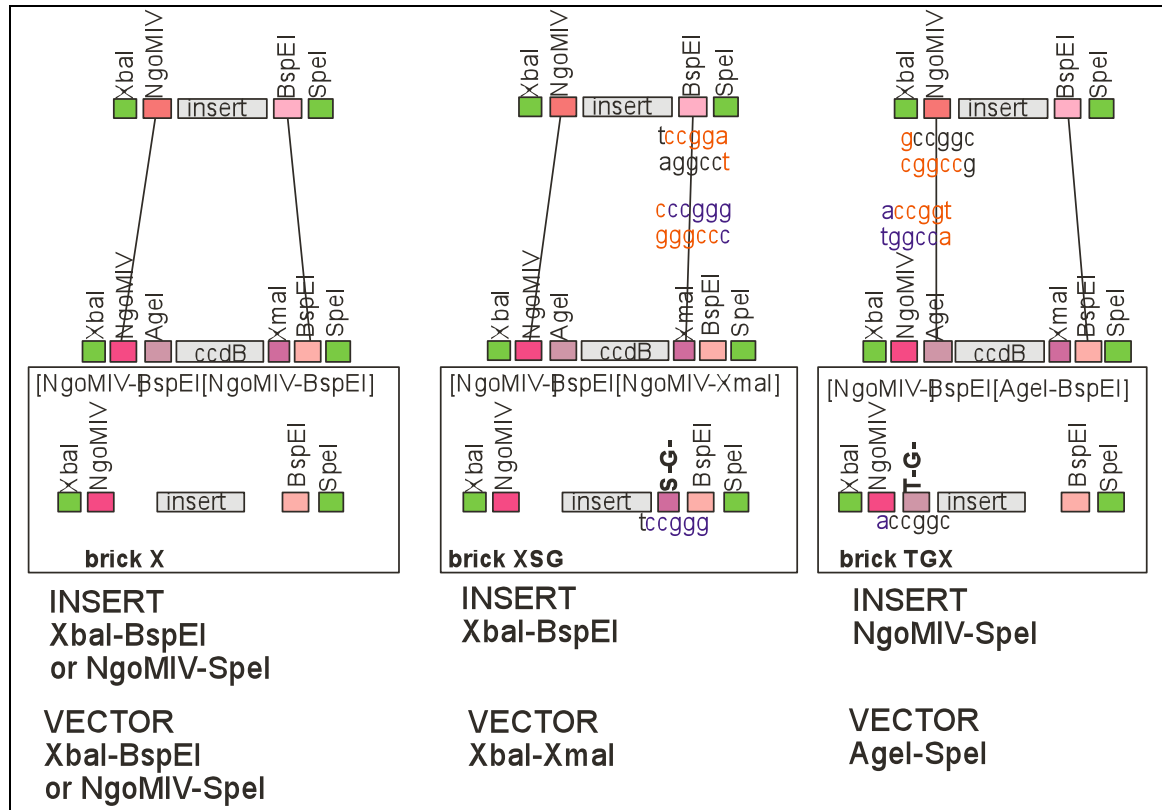
Schematic presentation of three different cloning strategies in multiple-cloning site of **BB-NIC-II vector**.

Note: Each type of cloning leaves different extension of amino acids that could be used to extend scar/linker between parts after in-frame parts assembly.
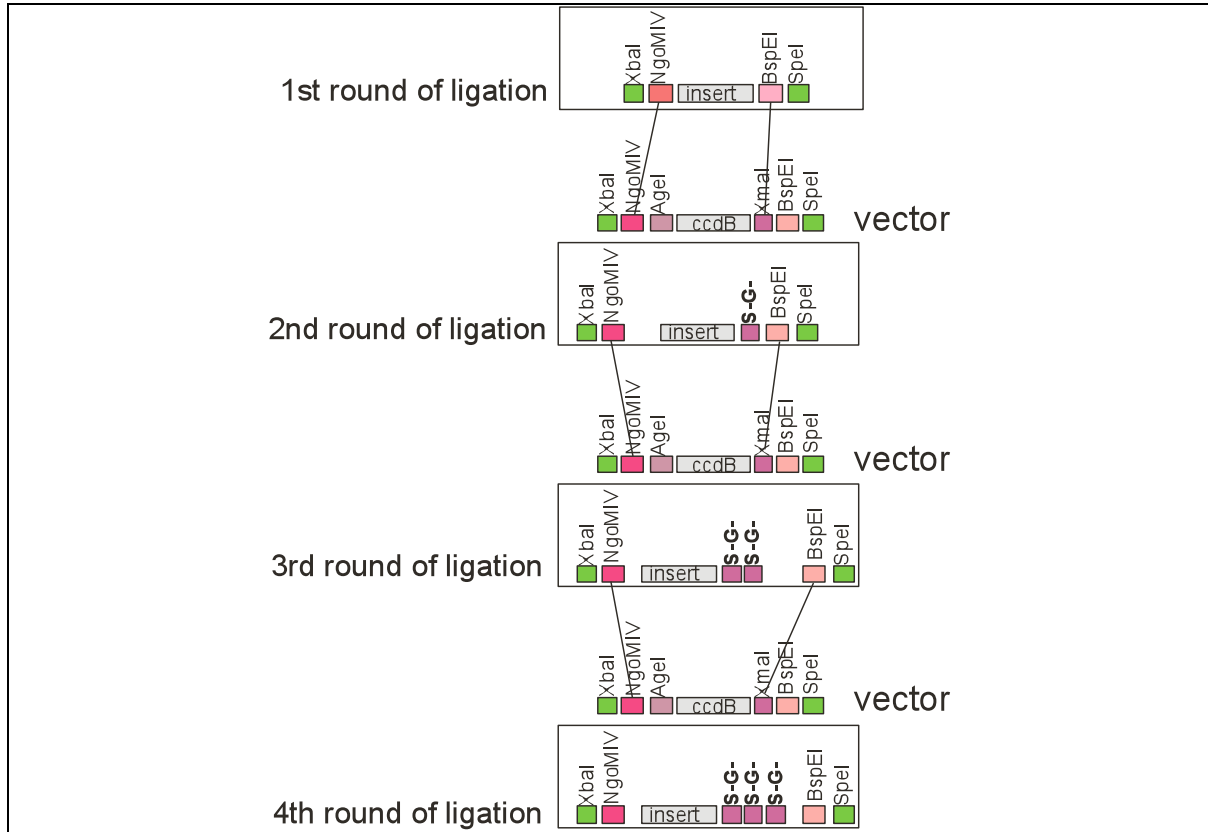
BioBrick-NIC-III:

Schematic presentation of four different cloning strategies in multiple-cloning site of **BB-NIC-III vector**.

Note: Each type of cloning leaves different extension of amino acids which could be used to extend scar/linker between parts after in-frame parts assembly.

4.3 Iterative linker extension

Schematic presentation of linker extension. Similar strategy could be used to incorporate other linker amino acids (see section 4.2 cloning for other options). With each round of cloning the insert gains two additional amino acid residues. Type of linker amino acids is defined with way of cloning (restrictions of vectors).

4.3   Assembly strategy

Assembly of two parts with linker extensions leads to BioBricks differing in linker size and composition.
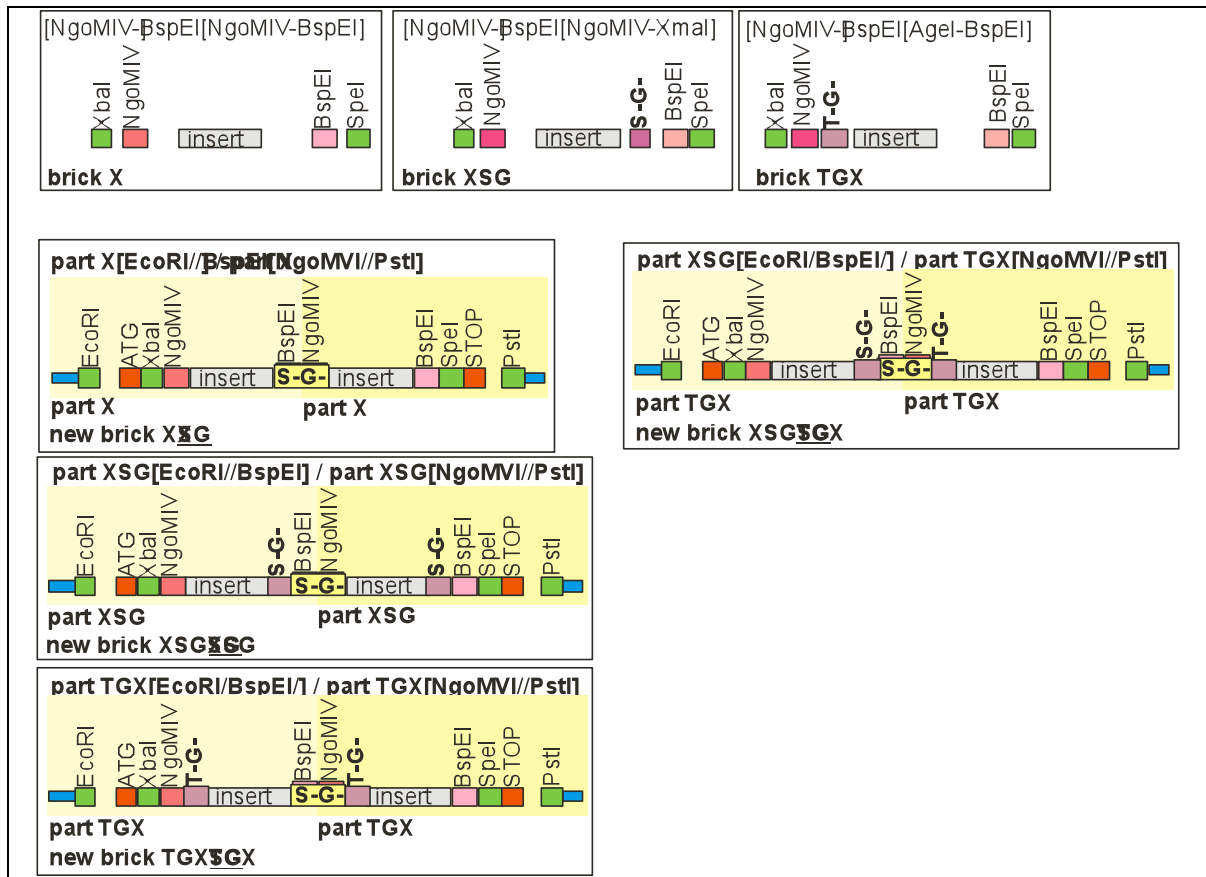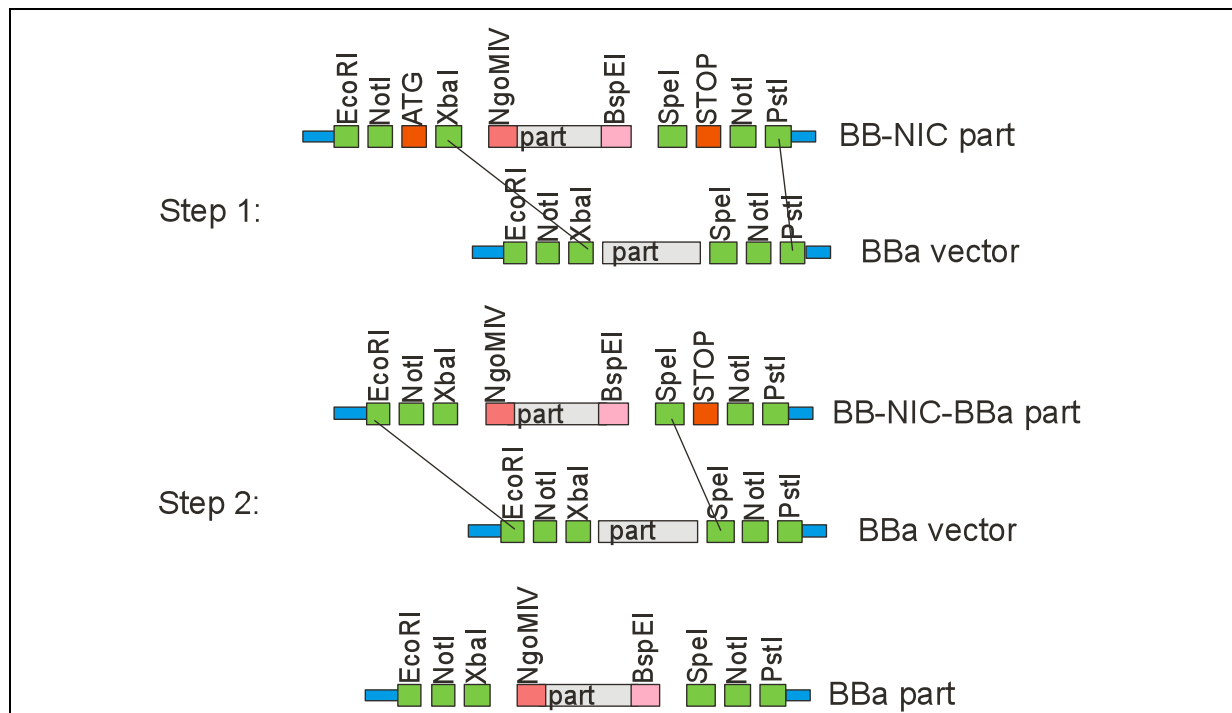
Schematic presentation of parts assembly.

4.4 Compatibility

Each part suited for cloning into standard BioBrick vector could be cloned also into BB-NIC vectors. The part according to the standard Biobrick vector could be cloned into BB-NIC vectors using *Xba*I, *Spe*I restriction sites with the following limitation that no linker extension strategy can be applied for standard parts.

Transfer of parts from BB-NIC vectors to BBa vector or any other BB vector (e.g. Assembly standard 23) carrying *Eco*RI/*Xba*I//*Spe*I/*Pst*I restrictions can be done in two steps.

Schematic presentation of part transfer from BB-NIC into BBa and alike vectors.

Two steps of cloning are required for directional cloning.

Step 1: Ligation of part from BB-NIC into BBa both cut with XbaI/PstI.

Step 2: Ligation of part from intermediate into BBa both cut with EcoRI/SpeI.

5   Summary

- BBF RFC 36 is an extension of BBF RFC 25 and compatible with BBF RFC 10.
- It enables in-frame fusion of protein parts and linker generation between in-frame ligated coding sequences.
- The BBF RFC 36 generates benign protein scar/scars similar to those of BBF RFC 25.
- The fusion format is compatible with the established BioBrick cloning protocols preserving standard restriction sites of prefix and suffix.
- Four new restriction sites are added in multiple-cloning site. Restriction enzymes could be heat inactivated. The blunt-cutting isochizomer of *Ngo*MIV (*Nae*I) and *Xma*I (*Sma*I) allows possibility of directional cloning.
- Enables stand-alone protein expression.

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Details are described also on: http://2009.igem.org/Team:Slovenia/Biobrick