

# BBF RFC 24: Conversion of Freiburg (Fusion) Biobricks to the Silver (BioFusion) format

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## 1 Purpose

This Request for Comments (RFC) describes a strategy for converting Bio-bricks from the Freiburg (aka Fusion) format to the Silver lab (aka BioFusion) format.

## 2 Relation to other BBF RFCs

BBF RFC 24 does not update or replace any earlier BBF RFC.

### 3 Copyright Notice

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## 4 Background

The original assembly standard for biological parts (BBF RFC 10) cannot be used for the construction of protein fusions. Different modifications have been proposed to overcome this problem [1]. Two of these alternative formats are relatively widely used:

(1) The Silver lab BioFusion format (BBF RFC 23) defines the following prefix and suffix sequences:

5' GAATTTC GCGGCCGCTCTAGA ...part... ACTAGT A GCGGCCGCTGCAG 3'  
 EcoRI NotI XbaI SpeI NotI PstI

(2) The Freiburg Fusion format defines the following prefix and suffix sequences:

```
...part... ACCGGT TAAT ACTAGT A GCGGCCG CTGCAG 3'  
AgeI * SpeI NotI PstI
```

Fusion and BioFusion parts can be coupled through their XbaI and SpeI restriction sites following the original standard assembly (RFC 10). However this introduces a frameshift between the two parts and retains the stop codon at the 3' end of the Fusion part. It is therefore not possible to mix the two different formats into one functional protein fusion.

This RFC introduces a strategy to transfer a Freiburg/Fusion part into a mixed format that is compatible with both the Fusion and the Silver/BioFusion assembly.

## 5 Relevant Parts

- CrossFusion plasmid pSB1AC3X = BBa\_J18904
- CrossFusion plasmid pSB1AK3X = BBa\_J18905
- CrossFusion plasmid pSB1AT3X = BBa\_J18906

All three plasmids are available from the MIT parts registry as well as from the author.

## 6 CrossFusion conversion vectors

I have constructed three conversion vectors with a modified Freiburg prefix / suffix that bring a Freiburg/Fusion part in frame with Silver/BioFusion parts and remove the stop codons between the AgeI and SpeI site. CrossFusion conversion vectors MUST adhere to the following format:

```
5' GAATTCTCGGGCCGC T TCTAGA GCCGGC ...part...  
EcoRI NotI XbaI NgoMIV  
NaeI  
  
...part... ACCGGT ACTAGT A GCGGCCG CTGCAG 3'  
AgeI SpeI NotI PstI
```

Each CrossFusion conversion vector SHOULD contain the ccdB negative selection cassette (part ID BBa\_P1010) to facilitate sub-cloning without gel purification. The vector SHOULD be provided in three variants with different antibiotic resistance markers to allow for 3A assembly. That means, each variant SHOULD feature the Ampicillin resistance and one additional resistance marker:

- pSB1AC3X: Ampicillin + Chloramphenicol
- pSB1AK3X: Ampicillin + Kanamycin
- pSB1AT3X: Ampicillin + Tetracycline

The new vectors – pSB1AC3X, pSB1AK3X, pSB1AT3X – are derived from the standard pSB1AC3, pSB1AK3, and pSB1AT3 construction plasmids.

## 7 Method

Restriction / ligation with AgeI + NaeI transfers Fusion parts into a CrossFusion conversion vector which can then be used for normal BioFusion cloning (at the cost of adding Thr Gly before and after the part). NaeI is an isoschizomer to NgoMIV but generates blunt ends which should allow for a directional transfer.

The conversion is then performed as follows:

1. digest Freiburg/Fusion part with NaeI and AgeI restriction enzymes
2. digest appropriate conversion vector with NaeI and AgeI
3. ligate the two restriction products
4. transform E. coli cells with the ligation reaction
5. plate transformants on selective medium – select for the unique resistance marker of the conversion plasmid
6. screen colonies for insert by colony PCR and sequence positive clones

The conversion vector SHOULD feature a resistance marker that is lacking from the original Fusion vector. For example, let's assume the original Freiburg Biobrick was shipped in the vector pSB1AC3F (part ID BBa\_J18904). The part could then be transferred into pSB1AK3X using Kanamycin-selective medium, or into pSB1AT3X using Tetracycline-selective medium.

A gel purification of the restriction products is not needed, thanks to the selection by ccdB against the “empty” conversion vector and the antibiotic selection against the source vector.

## 8 Discussion

Freiburg/Fusion, Silver/BioFusion (BBF RFC 23) and classical/BBa (BBF RFC 10) Biobrick parts can, in fact, be freely assembled with each other. However, the standard assembly (BBF RFC 10) leaves a Stop codon between the parts and introduces a frame shift between Silver and Freiburg formatted Biobricks. Such a mixed assembly can therefore not be used for the construction of fusion proteins. Nevertheless, even without any conversion, it is still possible to mix, for example, promoter or terminator parts in BBa or Silver format with Freiburg-formatted protein parts. To this end, one would first use the Freiburg assembly for the construction of a full-length protein from modular parts and then switch to the classic assembly for adding the BBa or Silver parts.

This RFC describes conversion vectors which, moreover, allow to integrate Freiburg formatted Biobricks (or assemblies thereof) directly into fusions proteins that are otherwise assembled from Silver formatted biobricks. It becomes thus possible to, for example, fuse a GFP protein from the Silver lab with a purification or localization tag from the Freiburg iGem team. However, this conversion only works in one direction. The resulting fusion construct contains internal AgeI and NgoMIV restriction sites and can no longer be used in Freiburg-style assemblies. Any further assemblies need to be performed with the classic set of enzymes as described in RFC 10.

The conversion adds a Thr-Gly pair of amino acids to both sides of the Biobrick part. Protein parts are often connected by flexible linkers and the additional 2 amino acids should, in many cases, not pose a problem.

This RFC does not offer any solution for a conversion in the opposite direction, that is, from Silver to Freiburg format.

## 9 Further developments

The conversion vectors have been available since 2008 but the method described in this RFC still needs to be tested. Please document your success at using this method!

Once the strategy has been put to a test, this RFC should be superseded by a new RFC with a detailed protocol.

## 10 Authors' Contact Information

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## References

- [1] Raik Grünberg and the Synthetic Biology Community. Overview over existing and proposed biobrick formats. [http://openwetware.org/wiki/The\\_BioBricks\\_Foundation:Standards/Technical/Formats](http://openwetware.org/wiki/The_BioBricks_Foundation:Standards/Technical/Formats). Available from World Wide Web: [http://openwetware.org/wiki/The\\_BioBricks\\_Foundation:Standards/Technical/Formats](http://openwetware.org/wiki/The_BioBricks_Foundation:Standards/Technical/Formats).