

BBF RFC 20: Constraint Relaxation of RFC 10 for Assembling Standard Biological Parts

Austin Che
rfc@austinche.name

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1 Purpose

This Request for Comments (RFC) modifies the assembly standard for biological parts proposed in BBF RFC 10 by replacing PstI with SbfI and dropping other sequence constraints. This change relaxes the constraints on the part sequence while maintaining compatibility with existing parts that use RFC 10.

2 Relation to other BBF RFCs

This RFC replaces BBF RFC 10 (Draft standard for Biobrick Biological Parts) by Tom Knight.

3 Copyright Notice

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4 Parts

4.1 Allowed Sequences

All parts **MUST NOT** contain the recognition sequences for the following four restriction enzymes:

- **EcoRI**: GAATTC
- **XbaI**: TCTAGA
- **SpeI**: ACTAGT
- **SbfI**: CCTGCAGG

Note that existing parts that conform to BBF RFC 10 also conform to the requirements of this RFC as the recognition sequence for SbfI includes the PstI recognition sequence. This RFC does not make a recommendation about the other restriction sites specified in BBF RFC 10 (NotI, PvuII, XhoI, AvrII, NheI, SapI).

If the SbfI sequence is present in a sequence, it is **RECOMMENDED** that the CTGCAG site be mutated so that both the SbfI and PstI sites are destroyed. By replacing PstI with SbfI, the expected number of mutations needed in a random sequence drops from 1 in $4^6 = 4096$ to 1 in $4^8 = 65536$. Thus, it is 16 times less likely that mutagenesis will be required to remove a restriction site internal to a part.

4.2 Part Format

4.2.1 Prefix Sequence

To allow the assembly of ribosomal binding sites with protein coding sequences, the prefix used for coding regions is distinguished from the sequence for non-coding parts.

For non-coding parts (the default) the part **MUST** contain an EcoRI site followed by a XbaI site 5' of the part. For compatibility with RFC 10, it is **RECOMMENDED** that the following sequence be used: **GAATTC GCGGCCGC T TCTAGA G**

For parts coding for proteins, the part **MUST** contain an EcoRI site followed by the sequence **TCTAG** immediately 5' to the ATG start codon of

the part. For compatibility with RFC 10, it is RECOMMENDED that the following sequence be used: **GAATTC GCGGCCGC T TCTAG**. Parts containing start codons other than ATG must be modified to use ATG as the start codon.

4.2.2 Suffix Sequence

Following the 3' end of the part, all parts MUST contain a SpeI site followed by a SbfI site. For compatibility with RFC 10, it is RECOMMENDED that the following sequence be used: **T ACTAGT A GCGGCCG CCTGCAGG**

5 Plasmids

All parts MUST be in plasmids that do not contain extra sites for any of the enzymes EcoRI, XbaI, SpeI, or SbfI, other than the sites found around the part sequence. It is RECOMMENDED that plasmids do not contain any PstI sites except for the one within the SbfI site.

6 Assembly

To assemble two parts according to the standard in this RFC, it is RECOMMENDED that (1) the prefix part be digested with EcoRI and SpeI, (2) the suffix part be digested with XbaI and SbfI, and (3) the destination plasmid be digested with EcoRI and SbfI. A three-way ligation of the two parts and destination plasmid will form a new part of the same form as the original parts.

6.1 Compatibility with RFC10

As this RFC is a relaxation of the requirements found in RFC 10, parts conforming to this standard (“new parts”) and parts conforming to RFC 10 (“legacy parts”) can be assembled with each other.

All assemblies involving either new or legacy parts SHOULD use a destination plasmid conforming to this RFC. The plasmid SHOULD be digested with EcoRI and SbfI. The prefix part, whether a new or legacy part, SHOULD be digested with EcoRI and SpeI. If the suffix part is a legacy part, it SHOULD be digested with XbaI and PstI. If the suffix part is a new part,

it SHOULD be digested with XbaI and SbfI. Other than the difference in enzyme used to digest the suffix part, the assembly procedures when mixing legacy and new parts is identical.

7 Experimental Considerations

All four of the enzymes described here are commercially available. For example, NEB sells EcoRI-HF, XbaI, SpeI, and SbfI-HF. NEB has been simplifying their restriction system by recommending NEBuffer 4 for as many of their enzymes as possible. However, the previous PstI enzyme only shows 50% activity in NEBuffer 4. With this new set of enzymes, all four enzymes show 100% activity in NEBuffer 4.

SbfI has no issue with possible interfering methylation from EcoKI, EcoBI, dam, or dcm methylases.

SbfI is more expensive and less commonly available than PstI. SbfI is about the same price as SpeI. If at any point, the enzyme SbfI ends up being a worse enzyme, such as being too expensive, or does not digest as well, PstI can be substituted back for the majority of parts. Thus, for parts that do not have a PstI site, there is minimal risk to follow this standard over RFC 10.

8 Summary

It is RECOMMENDED that all new parts be designed for this standard. New plasmids will need to be designed also and it is RECOMMENDED that all future assemblies use these new plasmids. All new parts and plasmids conforming to this standard can be easily mixed with legacy parts of the RFC 10 standard. For assemblies where the suffix part conforms to this standard, the resulting assembled part will automatically conform to this standard. Thus, there is a clear and easy migration path from the existing set of RFC 10 parts.