BBF RFC 54: Abbreviated BioBrick Prefix and Suffix for More Efficient Primer Design

Teoh Shao Thing, Shuhei Yasumoto, Tadashi Nakamura, Takahiro Saka, Kousuke Torigata, Takino Rie, Saya Kakuda, Lin Youfeng, Toshiyuki Otake, Yuki Miyatake, Hirayama Ikumi, Takuro A. Kagaya, Naoaki Ono, Donal Stewart, John Roger Wilson-Kanamori, Meng Lu, William Rostain, Maria Kowal, Richard Partridge-Hicks, Sarah Hunt, Marta Bereska, Hannah Fraser, Matthew Coombes, Damian Barnard, Alistair Elfick, Chris French

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1. Purpose

This Request for Comments (RFC) modifies the assembly standard for biological parts proposed in BBF RFC 10 by removing the NotI restriction site from the BioBrick Prefix and Suffix.

2. Relation to other BBF RFCs

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

3. Copyright Notice

4. Motivation

BBF RFC 10 requires that a Prefix and Suffix be added to any DNA sequence registered and submitted as a BioBrick Part. The Prefix contains, in 5' to 3' order, the restriction enzyme recognition sequences of EcoRI (GAATTC), NotI (GCGGCCGC) and XbaI (TCTAGA), while the Suffix contains the SpeI (ACTAGT), NotI, and PstI (CTGCAG) recognition sequences.

We have found the NotI site to be unnecessary and even inconvenient. In addition to having no discernable function in the commonly used Standard Assembly and 3A Assembly, its existence is not immediately apparent to new BioBrick users. In fact,

the NotI site is not mentioned in the Registry of Standard Parts' Help page on BioBrick Prefix and Suffix.

When incorporating the Prefix and Suffix into primers for PCR cloning, the following problems were observed:

a) The NotI site adds unnecessary length to the Prefix and Suffix, making them hard to keep within the usual recommended range of 15-25 bases. In addition it drives up the cost of primers as cost per base typically increases when the primer exceeds a certain length (around 30 bases).
b) It is purely composed of G and C bases, greatly increasing the primers' GC content. This, together with the length, often increases the Tm of the primers beyond the recommended 55-65℃.
c) As a palindromic sequence it has high self-complementarity, leading to easy formation of primer self- and heterodimers. The length of the sequence (8 bases) compounds this problem. This may adversely affect the reaction efficiency.

One procedure, currently in use at French lab in Edinburgh University and by the iGEM team of Osaka University, is to PCR clone parts for internal use without the NotI sites, only adding in the NotI site for parts to be submitted to the Registry. While this is a satisfactory workaround, reworking the parts for submission is a needless waste of effort as the NotI serves no discernable purpose.

5. Parts

5.1 Allowed Sequences

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

EcoRI	GAATTC
XbaI	TCTAGA
SpeI	ACTAGT
PstI	CTGCAG

This RFC does not make a recommendation about the other restriction sites specified in BBF RFC 10 (NotI, PvuII, XhoI, AvrII, NheI, SapI).

Note that the set of requirements of this RFC is a subset of that of RFC 10 – existing parts conforming to BBF RFC 10 also conform to the requirements of this RFC.

5.2 Part Format

5.2.1 Prefix Sequence

The prefix sequences for coding parts and non-coding parts are distinguished as follows. For *non-coding parts* (the default), the part MUST contain an EcoRI site followed by an XbaI site 5' of the part. It is RECOMMENDED that the following prefix be used: GAATTC CT TCTAGA G.

To allow the upstream attachment of ribosomal binding sites at sufficient proximity, *coding part* prefixes MUST contain an EcoRI site followed by the sequence TCTAG immediately 5' to the ATG start codon. It is RECOMMENDED that the following prefix be used: GAATTC CT TCTAG. Parts beginning with start codons other than ATG MUST be modified to use ATG as the start codon.

5.2.2 Suffix Sequence

Following the 3' end of the part, all parts MUST contain a SpeI site followed by a PstI site. It is RECOMMENDED that the following sequence be used: T ACTAGT AG CTGCAG.

6. Plasmids

All parts MUST be in plasmids that do not contain extra sites for any of the enzymes EcoRI, XbaI, SpeI or PstI, other than the sites found in the prefix and suffix.

7. Assembly

Assembly of two parts can be performed using either the original BioBrick Standard Assembly or the 3A Assembly method.

7.1 Compatibility with RFC 10

All parts conforming to BBF RFC 10 are compatible for assembly with parts conforming to this RFC.

8. Experimental Considerations

The shorter Prefixes and Suffixes specified by this RFC will be of great convenience to labs that routinely produce new parts by PCR. No new restriction enzymes are needed for assembly, and parts conforming to RFC 10 are all compatible with the more relaxed requirements of this RFC.

9. Summary

It is RECOMMENDED that the NotI sites be omitted from the Prefix and Suffix during the design of new parts. There is no need to change existing parts conforming to BBF RFC 10 as those parts already conform to the requirements of this RFC.

10. Authors' Contact Information

Teoh Shao Thing	shaothing@yahoo.com
Shuhei Yasumoto	shuhei_yasumoto@yahoo.co.jp
Tadashi Nakamura	tadasi.nakamura@gmail.com
Takahiro Saka	takahiro9bttf@yahoo.co.jp
Kousuke Torigata	colt_m16a4@yahoo.co.jp
Takino Rie	doudemo-iinosa@hotmail.co.jp
Saya Kakuda	kakuda150@gmail.com
Lin Youfeng	yuto@w4.dion.ne.jp
Toshiyuki Otake	viva_softbank_hawks@yahoo.ne.jp
Yuki Miyatake	million.miles.miles.away@gmail.com
Hirayama Ikumi	eating218sobabouro@yahoo.co.jp
Takuro A. Kagaya	tkagaya@bio.sci.osaka-u.ac.jp
Naoaki Ono	nono@ist.osaka-u.ac.jp
Donal Stewart	donal.stewart@ed.ac.uk
John Roger Wilson-Kanamori	s0458094@sms.ed.ac.uk
Meng Lu	s0971303@sms.ed.ac.uk
William Rostain	s0789154@sms.ed.ac.uk
Maria Kowal	s0801029@sms.ed.ac.uk
Richard Partridge-Hicks	s0784083@sms.ed.ac.uk
Sarah Hunt	sarah0674115@hotmail.co.uk
Marta Bereska	martabereska@gmail.com
Hannah Fraser	hpf2102@columbia.edu
Matthew Coombes	contradictory@hotmail.co.uk
Damian Barnard	dkbarnard@gmail.com
Alistair Elfick	Alistair.Elfick@ed.ac.uk
Chris French	C.French@ed.ac.uk