Dear iGEM judging team,

I am writing on behalf of the Slovenia iGEM team to request a variance for an improved BioBrick standard. This year, we will be trying to contribute parts to registry optimized for protein/peptide in frame ligation of parts. The specific added property of our upgraded standard is that it allows the option to modify the length of the linker between parts, which is often very important for the proper function of fusion protein and particularly for the construction of biomaterials. Our standard is completely compatible with BB standard as it results in the same outside restriction sites. We are describing our standard in more details as other teams may want to use it and we can provide them with already tested vectors. Standard will also be described on our wiki.

For a multitude reasons we would like to work with the upgrade of 2007 Freiburg iGEM proposed standard (Assembly standard 25). The upgrade includes altered multiple-cloning site which enables friendly scar after part ligation and simple extension of linker between parts.

Advantages:

in-frame fusion of protein parts
benign protein scar/scars
preserving standard restriction sites of prefix and suffix
four new restriction sites are added in multiple-cloning site
heat inactivation??
no Dam methylation problem for XbaI
stand-alone protein expression
full BBb compatibility and
blunt-cutting isochizomer of NgoMIV (NaeI) and XmaI (SmaI) possibility of directional cloning with two restriction enzymes enables part transfer between different formats and other potentially interesting transfer reactions

Disadvantages:

unexpected site effects for users not aware of different prefix/suffix
N-parts could be assembled with different enzyme combination
not compatible to BioFusion format (frame shift; stop codon)
not compatible to BBb format (Berkeley format)
additional limitations on the nucleotide sequence to avoid additional restriction sites

Here we describe sequence properties of modified vector BioBrick-NIC-II. All sequences defined herein are specified in the 5' to 3' direction.

A.   BioBrick-NIC-II originates from standard Biobrick vector. The difference is in multiple cloning sites.

The main reasons for changing multiple cloning sites are:

(i) Defined position of promoter and terminator from coding sequence coded with part or parts, which ensures efficient transcription and translation of protein; and

(ii) Additional sequences linked to parts, which could be exploited when linker is needed between combined parts.

Although most promoters and RBS and tags and terminators are currently specified as separate parts in the Registry, we will use a new design in which these elements are included within the vector, as we plan to prepare and isolate many proteins and this design will also decrease the number of required cloning steps and will be suesull for others desiring to prepare recombinant proteins in *E.coli*. We expect the new design will reduce the likelihood of unexpected functional composition problems between promoter / terminator and coding sequence.

The BioBrick-NIC-II vector is especially appropriate for cloning and combining parts coding short peptides.

The current assembly process requires certain sequence properties for the part and the surrounding DNA.

prefix: 5' gaattc(EcoRI) gcggccgc(NotI) promoter (invariant region) tctaga(XbaI) gccggc(NgoMIV) accggt(AgeI)

suffix: 5' cccggg(XmaI) tccgga(BspEI) terminator (invariant region) gcggccg(NotI) ctgcag(PstI)

B.   Multiple-cloning site
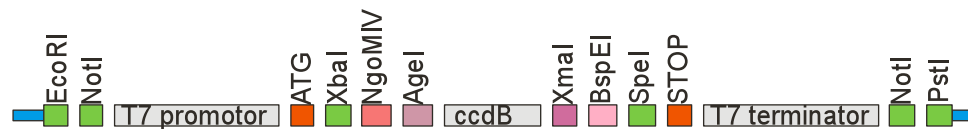
Three types of multiple cloning sites were constructed. First, between NotI site and XbaI sites of multiple cloning site of standard Biobrick vector a T7 promoter and ATG (star codon) with or without His-tag were inserted. Between SpeI and NotI sites a TAA (stop codon) and T7 terminator with or without His-tag were inserted. Between XbaI and SpeI site NgoMIV, AgeI, XmaI and BspEI sites were inserted. To simplify selection of positive clones containing ligated parts a CcdB domain was inserted between AgeI and XmaI sites.

1. MCS-I: N-His
gaattc(EcoRI) gcggccgc(NotI) T7-promoter-RBS-ATG Tyr-His-tag tctaga(XbaI) gccggc(NgoMIV) accggt(AgeI) ccdB-domain cccggg(XmaI) tccgga(BspEI) actagt(SpeI) STOP-T7-terminator gcggccg(NotI) ctgcag(PstI)

2. MCS-II: NC
gaattc(EcoRI) gcggccgc(NotI) T7-promoter-RBS-ATG tctaga(XbaI) gccggc(NgoMIV) accggt(AgeI) ccdB-domain cccggg(XmaI) tccgga(BspEI) actagt(SpeI) STOP-T7-terminator gcggccg(NotI) ctgcag(PstI)

3. MCS-III: C-His
gaattc(EcoRI) gcggccgc(NotI) T7-promoter-RBS-ATG tctaga(XbaI) gccggc(NgoMIV) accggt(AgeI) ccdB-domain cccggg(XmaI) tccgga(BspEI) actagt(SpeI) Tyr-His-tag STOP-T7-terminator gcggccg(NotI) ctgcag(PstI)

T7-promoter-RBS-ATG:
taatacgactcactatagggggaattgtgagcggataacaattcccctGtagaaataattttgtttaactttaaga aggaggtaaata ATG
Tyr-His-tag: TAT catcaccatcaccatcac
STOP-T7-terminator: TAA ctagcataacccccttggggcctctaaacgggG*ctagag*gggtttttttg

C.   Restrictions

Allowed sequences within BioBrick-NIC-II parts include any DNA sequence which does not contain the following subsequences:

|	
GAATTC	recognized by EcoRI restriction enzyme;
CTGCAG	recognized by PstI restriction enzyme;
GCGGCCGC	recognized by NotI restriction enzyme;
ACTAGT	recognized by SpeI restriction enzyme;
TCTAGA	recognized by XbaI restriction enzyme;
GCCGGC	recognized by NgoMIV restriction enzyme;
TCCGGA	recognized by BspEI restriction enzyme;
CCCGGG	recognized by XmaI restriction enzyme;
ACCGGT	recognized by AgeI restriction enzyme.

D.   Cloning

1. BioBrick-NIC-II Suffix: Each BioBrick-NIC-II part must contain precisely this sequence immediately following the 3' end of the part: **TCCGGA ACTAGT** (note: if constructing a primer, this sequence must be reverse complemented.)

2. BioBrick-NIC-II Suffix: Each BioBrick-NIC-II part must contain precisely the following sequence immediately 5' of the part: **TCTAGA GCCGGC**
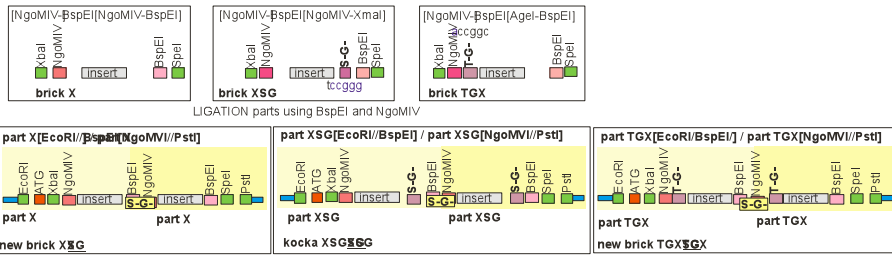
Part could be cloned into multiple cloning sites of **BioBrick-NIC-II** vector on four different ways:

1. Into XbaI and SpeI restriction site of multiple cloning sites.
2. Into NgoMIV and BspEI restriction site of multiple cloning sites.
3. Into NgoMIV and XmaI restriction site of multiple cloning sites. With this ligation two additional amino acids, Ser and Gly, are added on C terminal site of part.
4. Into AgeI and BspEI restriction site of multiple cloning sites. With this ligation two additional amino acids, Thr and Gly, are added on N terminal site of part.
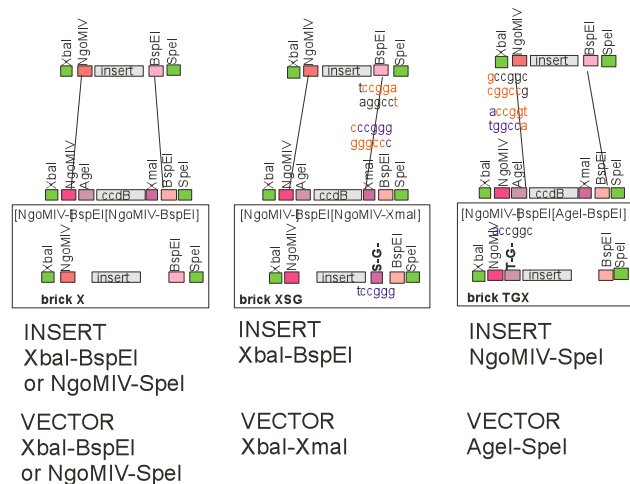
E.   Assembly Scars

1. With using XbaI PstI and EcoRI SpeI restriction sites for combining two parts a scar coding for D and R is formed.

2. With using NgoMI PstI and EcoRI BspEI restriction sites for combining two parts a scar coding for Ser and Gly is formed.

[NgoMIV-BspEI[NgoMIV-BspEI]

XbaI
NgoMIV
insert

BspEI
SpeI

brick X

[NgoMIV-BspEI[NgoMIV-XmaI]

XbaI
NgoMIV
insert

S-G-

BspEI
SpeI

brick XSG

tccggg

[NgoMIV-BspEI[AgeI-BspEI]

accggc

XbaI
NgoMIV
T-G-
insert

BspEI
SpeI

brick TGX

LIGATION parts using BspEI and NgoMIV

part X[EcoRI//BspEI[NgoMVI//PstI]

EcoRI
ATG
XbaI
NgoMIV
insert
S-G-
insert
BspEI
NgoMIV
BspEI
SpeI
PstI

part X   part X

new brick XSG

part XSG[EcoRI//BspEI] / part XSG[NgoMVI//PstI]

EcoRI
ATG
XbaI
NgoMIV
insert
S-G-
S-G-
insert
BspEI
NgoMIV
S-G-
BspEI
SpeI
PstI

part XSG   part XSG

kocka XSGSGG

part TGX[EcoRI/BspEI/] / part TGX[NgoMVI//PstI]

EcoRI
ATG
XbaI
NgoMIV
T-G-
insert
S-G-
insert
BspEI
NgoMIV
T-G-
BspEI
SpeI
PstI

part TGX   part TGX

new brick TGXSGX

F. Compatibility

Each part suited for cloning into standard Biobrick vector could be cloned also into vector BioBrick-NIC-II and vice versa. The part suitable for standard Biobrick vector could be cloned into BioBrick-NIC-II vector into XbaI, SpeI restriction sites in multiple cloning site.

G. Plasmid context

BioBrick-NIC-II vector is composed from similar elements (antibiotic resistance, origin of replication, ccdB domain) as standard Biobrick vectors with the exception of multiple cloning site.

H. Sequencing primers are identical as sequencing primers for standard Biobrick vectors and are:

VF2: TGCCACCTGACGTCTAAGAA;
VR: ATTACCGCCTTTGAGTGAGC.

I. Strains

The BioBrick-NIC-II vector and parts cloned into BioBrick-NIC-II vector could be cloned into the bacterial K-12 cloning strain (endA-). We recommend strains such as Top10, DH10B, and DH5a.