BBF RFC 21: BglBricks Assembly Standard

J. Christopher Anderson, John E. Dueber, Mariana Leguia, Gabriel C. Wu, Jonathan A. Goler, Adam P. Arkin, Jay D. Keasling 9/17/09

1. Purpose

The BglBricks standard has been developed as an alternative to the original XbaI/SpeI standard primarily to provide a solution to the issue of generating translational-fusion parts. When joined together in a standard assembly reaction, BglBricks parts contain a scar sequence of GGATCT between the two parts. This sequence conveniently translates as Gly-Ser in the zero frame, a commonly-used linker in protein engineering. BglBricks has previously been called the "BglBrick" standard, and is also referred to as Assembly standard 21. A consolidated description of the standard is available at: http://openwetware.org/wiki/Template:AndersonLab:BglBrick_Standard

2. Relation to other BBF RFCs

Similar standards have been introduced to address the problem of translational fusions:

BBF RFC 3: Restriction sites for the construction of fusion proteins

BBF RFC 23: A New Biobrick Assembly Strategy Designed for Facile Protein Engineering

BBF RFC 12: Draft Biobrick BB-2 standard for biological parts

BBF RFC 25: Fusion Protein (Freiburg) Biobrick assembly standard

3. Copyright Notice

Copyright (C) The BioBricks Foundation (2009). All Rights Reserved.

4. Definition of the Standard

The BglBrick standard is designed to guarantee that parts and vectors are compatible for standard assembly but imposes no explicit standards for composition. In essence, most but not all BglBrick plasmids encode an EcoRI-BglII-BglBrick_part-BamHI-XhoI cassette:

```
EcoRI BglII BamHI XhoI

5'-GAATTCatgAGATCT <part1> GGATCCtaaCTCGAG-3'

3'-CTTAAGtacTCTAGA <part1> CCTAGGattGAGCTC-5'

5'-GAATTCatgAGATCT <part2> GGATCCtaaCTCGAG-3'

3'-CTTAAGtacTCTAGA <part2> CCTAGGattGAGCTC-5'

$ scar

5'-GAATTCatgAGATCT <part1> GGATCT <part2> GGATCCtaaCTCGAG-3'

3'-CTTAAGtacTCTAGA <part2> CCTAGGattGAGCTC-5'

GlySer
```

Formally, for a double-stranded DNA sequence to be a BglBricks part its sequence **MUST** be flanked on the 5' end by "GATCT" and on the 3' end by "G" and lack BglII, BamHI, EcoRI, and XhoI restriction sites. This requirement guarantees that the restrictions sites will all be unique in any product plasmid. To be a BglBrick vector a DNA sequence **MUST** be flanked on its 5' end by "GATCC" and on its 3' end by "A", but all further rules describing a vector are special types of vectors. When a physical DNA is constructed, the composition of that DNA is one vector and one part, and the concatenation of the sequences will thus have unique BamHI and BglII sites. The one exception to this is the use of a "null vector." Concatenating a part with the null vector describes the cyclized product of the part, which has the effect of destroying the BamHI and BglII sites.

A BglBrick entry vector, such as pBca9145, is a special type of vector used for plasmid construction, maintenance, and assembly. It MUST contain a unique EcoRI site, no BamHI or BglII restriction sites, and MAY contain one XhoI site 5' to the EcoRI site. The nomenclature for a BglBricks plasmid SHOULD be "vector_name-part_name" and its sequence is the concatenation of its vector and part sequences. Each vector or part sequence SHOULD carry a unique name corresponding to its exact sequence. Derivatives of a BglBrick part or vector differing by even a single base SHOULD carry a unique name.

This minimal definition places no constraints on the location of other restriction sites, origins of replication, or antibiotic markers. Similarly there are no constraints on the length or sequence of the "prefix" and "suffix" regions (the sequence between EcoRI and BgIII and the sequence between BamHI and XhoI, respectively). Additional constraints on the design of BglBrick sequences are dynamic community-defined rules and should be viewed as sub-standards of BglBrick collected and described at http://openwetware.org/wiki/BglBrick or via the BBF. These sub-standards may include rules for the location of the start codon of a coding sequence, locations of other restriction sites, vector sets for specific standard assembly protocols, etc.

5. Author's Contact Information

J. Christopher Anderson jcanderson@berkeley.edu John E. Dueber dueber@gmail.com Mariana Leguia Gabriel C. Wu Jonathan A. Goler Adam P. Arkin Jay D. Keasling mariana.leguia@gmail.com gwu01@berkeley.edu jagoler@berkeley.edu aparkin@lbl.gov keasling@berkeley.edu