BBrickIt User manual

About

A BioBrick part can be defined as a DNA sequence (encoding a particular biological function) that conforms to a set of standard rules. The Registry of Standard Biological Parts consists of over 2000 standard parts, each of which can be assembled according to BioBrick assembly standards to create multi-component parts of increasing complexity and functionality (Shetty et al., 2008).
There are different types of assembly standards, namely BioBrick Standard Assembly (RFC 10), BioBrick BB-2 Assembly (RFC 21), Silver/Fusion Bricks (RFC 23), Freiburg Fusion Protein (RFC 25) and BioBrick MoClo (RFC 1000). (Registry of Standard Biological Parts, iGEM; Knight, 2007; Knight, 2008; Anderson et al., 2009; Philips et al., 2006; Grünberg et al., 2009). The Biobrick assembly standard RFC 10 is the most widely used assembly standard (Registry of Standard Biological Parts, iGEM). Each year hundreds of iGEM teams all over the world contribute and submit BioBrick compatible parts to the registry. The BioBrick standard has a set of stringent requirements for a part to be RFC 10 compatible:

RFC 10 Standards

Illegal and avoidable sites

Allowed sequences within Biobrick parts include any DNA sequence which does not contain the following subsequences:

Enzyme	Type	Sequence
EcoRI	Illegal	gaattc
XbaI	Illegal	tctaga
SpeI	Illegal	actagt
PstI	Illegal	ctgcag
NotI	Avoidable	gcggccgc

Prefix and Suffix

Each Biobrick part must contain the following sequence as suffix downstream of the 3' end of the part: (Knight, 2007)
T ACTAGT A GCGGCCG CTGCAG

The prefix used for coding regions is different from the sequence for non-coding Biobrick parts. This is to allow the construction of ribosomal binding sequences 5' of coding regions.

1.. For non-coding Biobrick parts this prefix must be placed 5' upstream of the part:
GAATTC GCGGCCGC T TCTAGA G

2. For Biobrick parts coding for proteins, the following sequence should be placed 5' upstream of the start codon (ATG) of the coding region:
GAATTC GCGGCCGC T TCTAG

Terminating

All parts containing start codons other than ATG must be changed to possess ATG as the start codon. Though not mandatory, it is highly recommended, that all coding regions terminate with in-frame TAATAA stop codons, replacing other stop codons (TGA, TAG) if needed. (Knight, 2007)

Program functions

This program performs the following functions:

If sequence contains illegal/avoidable sites

1. If the sequence to be contains any of the illegal or avoidable sites, the program will detect them and they will be highlighted.
2. As a replacement for such sequences, a new sequence is suggested, with some of the nucleotides in the restriction sites replaced based on the choice of the chassis organism.
3. This new, optimized sequence can then directly be BioBricked.

If sequence does not contain illegal/avoidable sites

1. The BioBrick Prefix is added to the sequence. If the sequence is non-coding, the prefix GAATTCGCGGCCGCTTCTAGAG is added upstream of the 5' end of the sequence. If the sequence is coding, the prefix GAATTCGCGGCCGCTTCTAG is stream of the start codon (either ATG or GTG) of the sequence
2. The BioBrick Suffix TACTAGTAGCGGCCGCTGCAG is added downstream of the 3' end of the sequence.
3. An additional 8 nucleotide sequence, GTTTCTTC (Shetty et. al. 2008), is added to both flanks of the BioBricked part, to allow cleavage by restriction endonuclease close to the end of linear DNA Fragments.
4. If the sequence is a coding sequence (CDS), the in-frame stop codon (TGA, TAG) will be changed to TAATAA, as per BioBrick Primer design criteria (Registry of Standard Biological parts: Primer Design; Knight, 2007).

Steps to Use

1. Download the file from our GitHub page: https://github.com/igemsoftware2018/Team_ICT_Mumbai
2. Read the GitHub documentation (ReadMe file) carefully before downloading. It provides comprehensive instructions on which file to download for Mac or Windows and its subsequent usage.

3. On the home screen, select your Chassis Organism from the radio buttons on the right.
4. Paste your sequence in box at the top that says “Paste your sequence here”.
5. Select the checkbox at the bottom of the entry box if your sequence is a coding sequence, leave it empty if it is not.
6. Click “BioBrick!” if the sequence is in correct order, else click “Reverse complement and BioBrick” if you want to perform the operations on the reverse complaint of the entered sequence.
7. If your part contains any illegal restriction enzyme binding sites, it will be highlighted in an output box on the bottom left of the window and the highlighted restriction sites with an indicative legend.
8. A box on the bottom right will suggest the new, optimized sequence for selected organism. If you wish to proceed with that sequence, copy and paste it into top box and hit “BioBrick”.

References

1. *Idempotent Vector Design for Standard Assembly of Biobricks by Tom Knight*
2. *BBF RFC 9: Idempotent vector design for the standard assembly of Biobricks by Tom Knight, Randall Rettberg, Leon Chan, Drew Endy, Reshma Shetty, Austin Che*
3. *BBF RFC10: Draft standard for Biobrick biological parts by Tom Knight*
4. *BBF RFC 21: BioBricks Assembly Standard by Anderson et al.*
5. *A New Biobrick Assembly Strategy Designed for Facile Protein Engineering by Philips et al.*
6. *Fusion Protein (Freiburg) Biobrick assembly standard by Grünberg et al.*
7. *http://parts.igem.org/Help:BioBrick_Prefix_and_Suffix*
8. *Engineering BioBrick Vectors from BioBrick Parts; Shetty RP et. al.; J Biol Eng. 2008; 2: 5; DOI: 10.1186/1754-1611-2-5*
9. *http://parts.igem.org/Help:Primers/Design*