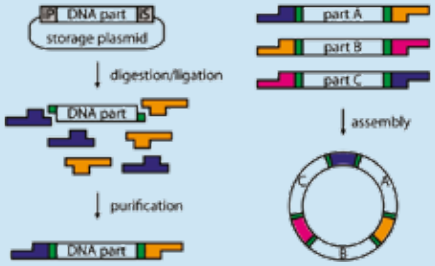
APPLICATION NOTE

BASIC: DNA ASSEMBLY

FASTER, MORE ACCURATE, MORE FLEXIBLE

B ioPart
A ssembly
S tandard for
I dempotent
C loning



BASIC is a fast, accurate and modular DNA assembly method for synthetic biology and general molecular biology applications. Created by researchers from Imperial College London's Centre for Synthetic Biology & Innovation[1], it provides a DNA assembly standard and a method, which uses oligo linkers to assemble DNA bioparts.
Biolegio offer all BASIC linkers and reagents required to perform BASIC assembly in a user-friendly format.

WE ♥ YOUR DNA

Biolegio

DNA ASSEMBLY COMPARISON TABLE

Method	Standards	PCR required	Forbidden restriction sites	Multipart assembly	Hierarchical Assembly?	Single tier assembly?	Single storage format	Automation friendly
Restriction-Ligation	Bespoke	Yes	Multiple	No	No	No	No	No
Gateway Cloning®	Gateway	No	1,2	No	No	No	Yes	No
BioBrick®	BioBrick	No	4	No	Yes	Yes	Yes	Yes*
Gibson Assembly®	Bespoke	Yes	None	Yes	No	No	No	Yes**
Ligase Cycling Reaction	Bespoke	Yes	None	Yes	Yes	No	No	No
Golden Gate	CIDAR, Greengate	No	1-4	Yes	Yes	No	No	Yes
BASIC	BASIC	No	1	Yes	Yes	Yes	Yes	Yes

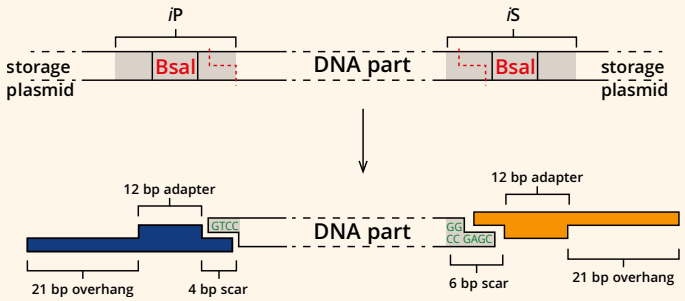
* The two-part rolling assembly required for BioBricks® means that transformation and mini-prep will be required for any assembly larger than two parts, which significantly limits the implementation of automation methods.

** Gibson Assembly® is available as an automated gene synthesis platform available from SGI-DNA Inc. It should be noted that this is intended for bespoke gene synthesis and assembly and cannot be used just for the assembly of user parts only.

BASIC combines the best features of the most popular methods while overcoming their limitations, creating a system that is fast, flexible and accurate. The new technique enables greater advances in research and offers a way to automate the design and manufacture of plasmids/ DNA constructs.

Unlike some systems that can only join two parts at a time, forcing the gene to be built in several, time consuming steps, BASIC enables multiple parts to be joined together at once. It is also 99 per cent accurate, compared to bespoke designs which usually have an accuracy of around 70 per cent.
BASIC DNA assembly is highly accurate for up to 7 parts.

Figure 1 | **BASIC workflow**



- 99% correct clones
- Takes just 1 hour of work over a single day
- All reagents provide by Biolegio

Industrial scale compatible

The standardisation and accuracy of the process means that it is ideally suited for use on an industrial scale. The method is already used in a high throughput automated process at SynbiCITE, the innovation and knowledge centre (IKC) based at Imperial College London which is promoting the adoption of synthetic biology by industry.

BASIC linker supply format

Each BASIC linker consists of 4 oligos and is made up of a suffix (orange) and prefix (blue) section. The BASIC linker suffix section will be ligated to the suffix overhang of a BASIC biopart and the BASIC linker prefix section will be ligated to the prefix overhang of a BASIC biopart.

Biolegio provides ready to use BASIC linkers in different package sizes and sets. These include an accompanying reagents pack containing all enzymes and buffers required to perform digestion/ligation, purification and final DNA assembly

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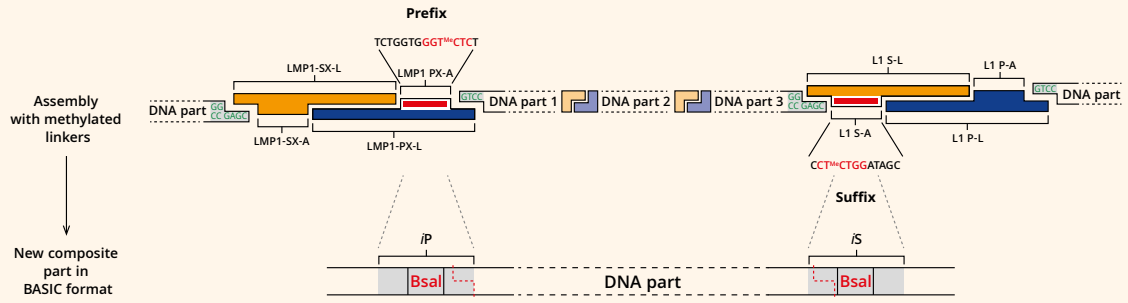
Figure 2 | Composite part in BASIC format



Each section is made up of one longer linker oligo (30-60 bases) and a shorter adapter oligo (12-20 bases). These 4 oligos are all **5' phosphorylated and HPLC purified**. Both, the suffix and prefix oligos are mixed and annealed each prior to usage in BASIC assembly.

Each BASIC linker will be provided lyophilised in 2 tubes, one for the prefix and one for the suffix linker section, which can then be easily reconstituted to working concentration by adding BASIC linker annealing buffer. Such a linker aliquot is sufficient for 40 BASIC reactions. Each BASIC reaction can be used to build multiple genetic constructs.

Biolegio provides ready to use BASIC linkers in different package sizes and sets. These include starter kits including methylated linkers and sets of linkers encoding RBS (specific and mixed libraries).

SET 1: BASIC Starter Set

BASIC Neutral Linkers

BASIC neutral linkers are purely connecting two adherent BASIC parts and their sequence is computationally optimised to be as neutral as possible by excluding functional sequence motifs. The BASIC standard defines two inward-facing BsaI recognition sites to release the parts from a storage vector, leaving a 4 bp scar on the prefix end and a 6 bp scar on the suffix. Digestion yields different 4 bp overhangs at the prefix and suffix, enabling end-specific ligation. Ligation of partially double-stranded oligonucleotide DNA linkers is performed simultaneously with BsaI digestion. Non-ligated oligonucleotide linkers are then removed by a purification step to yield Linker adapted parts. Final assembly is achieved by annealing the linker-adapted parts in an ionic buffer at elevated temperature. No ligase is required in the final step and the nicked plasmid generated is readily repaired *in vivo* following transformation.

BASIC Methylated Linkers

In many cases it is advantageous to assemble a limited number of parts together in a module and then combine different modules to create more complex systems or to reuse modules in different assemblies. This is possibly without the need to modify the existing single tier protocol by utilising linkers with a single methylated C to protect the BsaI site from digestion during the assembly process (fig. 2).

SET 2: BASIC RBS Linkers

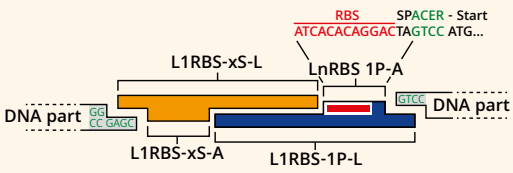
Flexible Gene Expression

BASIC linkers can be derived from neutral sequence space or encode functional elements to build more complex/ functional constructs. By encoding a Ribosome Binding Site (RBS) onto the prefix site of BASIC linkers, these can drive the expression of a downstream open reading frame. Different RBS sequences are encoded on the double stranded portion of the linker. The overlap sequence remains constant and provides a consistent 5' UTR. Different RBS linkers can be built by re-using the linker suffix section, while changing the sequences of the prefix adapter and linker oligo.

RBS Linker Families

We define RBS linker families as a group of RBS BASIC linkers that share the same upstream linker sequence (suffix section) and hence the same suffix linker/adapter oligos. This means each RBS linker family contains 2 standard suffix section oligos and 2 specific prefix site oligos for each RBS version of the RBS linker family (fig. 3).

Figure 3 | RBS linker family

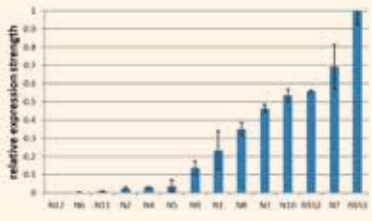


RBS identifier	Relative GFP expression
1	5 %
2	56 %
3	100 %
N-1	23 %
N-2	3 %
N-3	46 %
N-4	3 %
N-5	4 %
N-6	1 %
N-7	69 %
N-8	35 %
N-9	14 %
N-10	54 %
N-11	1 %
N-12	0 %

We currently offer 3 orthogonal RBS linker families with variable RBS sequences. Orthogonal means they can be used within the same BASIC assembly because their final assembly mediating overlap regions are distinct. For instance, the 3 RBS family linkers can be used to build a 3-gene operon expression plasmids from BASIC ORF parts in a single step.

The table above summarises RBS sequences we characterised and which can be used in any RBS linker family. From preliminary experiments, we also provide estimates for GFP expression relative to the strongest RBS (RBS-3). These relative expression levels will be different for each gene of interest and this RBS set is optimised to provide a good coverage of expression levels for any gene of interest (fig. 4).

Figure 4 | RBS ranking for sfGFP



SET 3: Mixed BASIC RBS linkers

In a typical application, BASIC users may want to work with a new gene of interest and like to identify RBS sequences providing discrete levels of relative expression strength (10%, 50%, 100% for instance). While there are bioinformatic tools available to predict RBS strength in a given context, the consensus in the field is that they are not reliable yet and in practise RBS sequences have to be screened for any given gene.
Experience shows the N1-12 RBS sequence family already provides a good range of expression strength, however obviously larger RBS libraries will offer more fine tuning capabilities. Rather than building 36 distinct BASIC constructs and identify the expression strength of each, mixed BASIC RBS linker pools were established. To this end we have 3 such mixed RBS pools available- NM12, NM24, NM36 containing RBS sequence N1-12, N1-24 and N1-36 respectively (fig. 5).

Figure 5 | GFP expression range for different mixed RBS linkers

References

1 Storch, M., Casini, A., Mackrow B., Fleming, T., Trewhitt H., Ellis T., Baldwin G.S.
BASIC: a new Biopart Assembly Standard for Idempotent Cloning provides accurate, single-tier DNA assembly for synthetic biology. ACS Synthetic Biology, 2015, DOI:10.1021/sb500356d

Biolegio

Contact details

Postal Address Biolegio BV
P.O. Box 91
6500 AB Nijmegen
(the Netherlands)

Visiting address & contact details
Lagelandseweg 56
6545 CG Nijmegen
(the Netherlands)

Phone: +31 (0)24 358 68 85
Fax: +31 (0)24 358 02 59
Email: info@biolegio.com
Web: www.biolegio.com

Please contact us for your local account manager or distributor.

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