basicsynbio and the BASIC SEVA collection: Software and vectors for an established DNA assembly method

Matthew C. Haines^{a,c,1}, Benedict Carling^b, James Marshall^b, Marko Storch^{c,1}, Paul Freemont^{a,c,d,1}

^a Department of Infectious Disease, Sir Alexander Fleming Building, South Kensington Campus, Imperial College London, SW7 2AZ, UK

^b Department of Bioengineering, Imperial College London, SW7 2AZ, UK

^c London Biofoundry, Imperial College Translation & Innovation Hub, London, W12 0BZ, UK

^d UK DRI Care Research and Technology Centre, Imperial College London, Hammersmith Campus, Du Cane Road London, W12 0NN, UK

¹ Correspondence: matthew.haines10@imperial.ac.uk, m.storch@imperial.ac.uk, p.freemont@imperial.ac.uk

Abstract

Standardized DNA assembly methods utilizing modular components provide a powerful framework to explore design spaces and iterate through Design-Build-Test-Learn cycles. Biopart Assembly Standard for Idempotent Cloning (BASIC) DNA assembly uses modular parts and linkers, is highly accurate, easy to automate, free for academic and commercial use, while enabling simple hierarchical assemblies through an idempotent format. These attributes facilitate various applications including pathway engineering, ribosome binding site tuning, fusion protein synthesis and multiplex gRNA expression. In this work we present basicsynbio, an open-source software encompassing a Web App (<https://basicsynbio.web.app/>) and Python Package (<https://github.com/LondonBiofoundry/basicsynbio>). With basicsynbio, users can access commonly used BASIC parts and linkers while robustly designing new parts and assemblies with exception handling for common design errors. Furthermore, users can export sequence data and create build instructions for manual or automated workflows. The generation of build instructions relies on the BasicBuild Open Standard which is easily parsed for bespoke workflows and is serialised in Java Script Object Notation for transfer and storage. We demonstrate basicsynbio by assembling a collection of 30 BASIC-compatible vectors using various sequences including modules from the Standard European Vector Architecture (SEVA). The BASIC SEVA collection encompasses plasmids containing six antibiotic resistance markers and five origins of replication from different compatibility groups, including a temperature-sensitive variant. We deposit the collection on Addgene under an OpenMTA agreement, making them available. Furthermore, these sequences are accessible from within the basicsynbio application programming interface along with other collections of parts and linkers, providing an ideal environment to design BASIC DNA assemblies for bioengineering applications.

Keywords: bioinformatics, DNA assembly, synthetic biology, software, cloning

Introduction

DNA assembly is an essential tool in Synthetic Biology and Life Sciences, required for building genetic designs and iterating through the Design-Build-Test-Learn cycle^{1,2}. A large repertoire of DNA assembly methods are available to researchers and the choice of a suitable method will depend on factors such as the freedom to include forbidden restriction sites or a need for high accuracy^{2,3}. Standardized and modular DNA assembly methods are ideal for high-throughput and hierarchical assemblies enabling the cost-effective generation of large numbers of constructs with high accuracy while encouraging the reuse of parts across designs^{2,4-7}.

BASIC DNA assembly is a standardized DNA assembly method which utilizes modular parts and linkers as functional units⁷⁻¹⁰. The method benefits from several desirable attributes including a single part storage format, and assembling up to 14 parts and linkers per round with > 90 % accuracy⁷. It is free for academic and commercial use and only requires the absence of one restriction enzyme site (BsaI). It is easy to automate the physical workflow¹⁰ and conduct hierarchical assemblies since parts don't require modification once in storage vectors and assemblies are ubiquitously returned with standardized flanking sequences. Both features are enabled by the underlying single-tier, idempotent format. This compares favourably with modular methods using Golden Gate assembly^{5,6}, where multiple restriction enzymes are utilised and assemblies not conforming to standard transcriptional units e.g., operons, are unsupported or prohibited. Notably, BASIC DNA assembly was successfully applied to several areas of Synthetic Biology and Life Sciences research including combinatorial pathway engineering^{3,11}, synthetic operon¹⁰ & sRNA circuit design¹², combinatorial gRNA expression for gene editing¹³, ribosome binding site (RBS) tuning and fusion protein engineering⁷. Now all facilitated by commercially available linkers.

In this work, we developed basicsynbio software with several aims. Firstly, we aim to make commonly used parts and linkers more accessible for users. Secondly, we aim to prevent assembly and part design mistakes by introducing exception handling. Thirdly, we aim to provide users with the ability to export a variety of data types for downstream building, validating, and sharing of assemblies. This extends and complements our previous work automating BASIC DNA assembly on a specific platform¹⁰. We demonstrate basicsynbio by

designing and exporting data for a collection of 30, BASIC-compatible vectors containing modules from the SEVA database^{14,15}. By building this collection and making it available we make applications requiring multiple plasmids or specific origins of replication more accessible for BASIC DNA assembly users.

Materials and methods

Preparation of BASIC linkers and parts

Apart from BSEVA_L1, all BASIC Linkers were acquired from Biolegio (BBT-18500) and prepped according to the manufacturer's instructions. Oligos for BSEVA_L1 (Supplementary Table S1) were ordered from Integrated DNA Technologies, Inc. and linker halves prepared as previously described⁹.

Unless specified, all plasmid DNA was prepped using Omega BIO-TEK E.Z.N.A.® Plasmid Mini Kit II according to the manufacturer's instructions. All plasmid DNA was quantified using Qubit™ dsDNA BR Assay Kit (Thermo Scientific™ Q32850).

Each BASIC SEVA vector is composed of three parts: T0 + marker part, ori + T1 part and mScarlet counter-selection cassette. Initially, each was either chemically synthesised or amplified from SEVA vectors¹⁵ with primers incorporating *iP* and *iS* sequences upstream and downstream, respectively. The resulting linear sequences were cloned into an appropriate vector, prior to prepping plasmid DNA. Specifically, T0 + marker parts were blunt cloned into pJET1.2 (Thermo Scientific™ K1231) according to the manufacturer's instructions. ori + T1 and mScarlet counter-selection cassette parts were assembled as described in the supplementary data (oris.gb & addgene_submission.pdf) using BASIC DNA assembly⁸. Constructs were plated on LB-agar (ForMedium) supplemented with 100 µg/mL carbenicillin and incubated at 30 or 37 °C prior to picking colonies and prepping plasmid DNA. All parts were sequence verified via Sanger Sequencing, using sequencing primers listed in Supplementary Table S1.

Assembly and validation of the BASIC SEVA collection

All assemblies were designed *in silico* using basicsynbio (Supplementary Data: addgene_submission.pdf). Echo instructions for the "Assembly reaction" step of the workflow and manual instructions for the entire workflow were exported (see Supplementary Data).

Clip Reaction and Magbead purification steps were implemented as described in the manual instructions (Supplementary Data: BASIC_SEVA_collection_v10_manual.pdf), transferring purified clip reactions to an Echo® Qualified 384-Well Polypropylene Microplate (Beckman 001-14555). Purified clip reactions were mixed by executing the `echo_clips_1.csv` script (Supplementary Data) on a Beckman Echo 525 Acoustic Liquid Handler, using a 96-well destination plate (Azenta Life Sciences 4ti-0960). ddH₂O and 10x assembly buffer solutions were transferred to the same destination plate by executing the `echo_water_buffer_1.csv` script with both solutions transferred from an Echo® Qualified Reservoir, 2x3 Well, Polypropylene Microplate (Beckman 001-11101). The destination plate containing assemblies was sealed with a PCR foil seal (Azenta Life Sciences 4ti-0550) and vortexed prior to incubating at 50 °C for 45 min. 25 µL NEB® 5-alpha Competent E. coli cells (C2987) were added to each assembly reaction on ice. Transformation reactions were incubated for 20 min on ice, followed by heat shock at 42 °C for 15 sec, recovery on ice for 2 min, the addition of 150 µL SOC media (ForMedium) and outgrowth at 30 °C for 2 hr. Cells were plated on LB-agar containing antibiotics as illustrated in Figure 4b. Plasmid DNA from pink colonies was prepped as described above for corresponding ori + T1 parts.

To verify the presence of the correct ori + T1 part, each BASIC SEVA vector was sequenced using the BSEVA_L1_overhang sequencing primer (Supplementary Table S1). The resulting data was analysed using cMatch¹⁶ to verify homology.

Availability of sequences

All BASIC SEVA plasmids were deposited on Addgene (Deposit 80391) and were made available under Addgene's OpenMTA agreement.

Results and Discussion

basicsynbio workflow

We conceived a typical workflow for users implementing basicsynbio (Figure 1a). Initially users would access collections of parts and linkers available from the basicsynbio Application Programming Interface (API), in addition to importing their own. These BasicPart and BasicLinker objects are combined initiating BasicAssembly objects representing assembled constructs. A key advantage of BASIC DNA assembly is its idempotency, meaning that BasicAssembly objects can

function as new BasicParts in subsequently larger constructs. basicsynbio facilitates this, enabling users to convert BasicAssembly objects into BasicParts, ready to initiate next-tier, larger BasicAssembly objects. Once the user has specified all the desired BasicAssembly objects, various data types are available to export (Figure 1a and Supplementary Figure S1). Users can export sequence data representing BasicAssembly and BasicPart objects in GenBank via the Web App or in formats supported by Biopython¹⁷ via the Python Package. Notably, all sequence features are preserved, maintaining feature annotations in the resulting assemblies for the identification of regions associated with a desired function. In addition to exporting sequence data, users can export build instructions, for instance instructions for manual or automated workflows e.g., manual instructions in pdf or instructions for a Beckman Echo liquid-handler.

To aid accessibility of existing core BASIC DNA assembly part and linker sequences, we include PartLinkerCollection objects, accessible from the API and containing instances of commonly used BasicParts and BasicLinkers. Notable collections are illustrated in Figure 1b, including BASIC_BIOLEGIO_LINKERS, BASIC_PROMOTER_PARTS and BASIC_SEVA_PARTS which contain all 65 commercially available Biolegio linkers, including linkers for 81 different untranslated region (UTR)/ribosome binding site (RBS) combinations, a collection of 60 inducible and constitutive promoters, insulated by different combinations of upstream terminators and downstream RiboJ sequences¹⁸ and a collection of 30 vectors derived from the Standard European Vector Architecture¹⁵, respectively. The latter collection of vectors was developed during this work. To aid the exploration of PartLinkerCollections, users can visualize individual parts and linkers via the Web App using SeqViz or DNAFeaturesViewer¹⁹ (Supplementary Figure S2 & S3). Different versions of a given PartLinkerCollection are supported enabling future updates where required. Furthermore, users can contribute new PartLinkerCollections as described in the online documentation²⁰. We hope this will encourage the BASIC DNA assembly user community to share collections of new part and linker sequences for different applications between labs and institutions.

In addition to the above PartLinkerCollections, users can import parts from local files or external sources and/or create new linkers using the API, greatly expanding the number of possible assemblies. Users may import parts specified in commonly used file formats such as FASTA,

GenBank and SBOL (Figure 1b & Figure 2). Furthermore, to aid the generation of new parts, users can automatically add required *iP* and *iS* sequences⁷ to the 5' and 3' ends of input DNA sequences, respectively. It is also possible to design primers to add *iP* and *iS* sequences to parts via overhang PCR with the aid of Primer3²¹. This enables cost-effective conversion of existing DNA sequences into a physical part, avoiding the need for *de novo* DNA synthesis. For a given linker, users can calculate the four oligonucleotides required to generate linker halves, an adapter and long oligonucleotide for each linker half^{7,9} (Figure 1b). This feature aids the generation of custom linkers required for specific applications or organisms.

For the successful implementation of BASIC DNA assembly, imported parts and designed assemblies must satisfy several conditions (Figure 1c). For instance, if the length of a part is significantly shorter than 100 bp, the linker-ligated part would be lost during the purification step of assembly. Additionally, internal BsaI sites are not allowed in BASIC parts and specific linkers can only be used once per assembly round, while BasicPart and BasicLinker objects must alternate, with equal numbers of each. Where a user designs an assembly that doesn't satisfy the above conditions, basicsynbio raises one or more exceptions preventing subsequent experimental failure, increasing robustness.

To implement the basicsynbio workflow illustrated in Figure 1a, users can utilize the open-source Python Package or Web App. Python iterator patterns²² combined with the basicsynbio package allow users to initiate large numbers of BasicAssembly objects programmatically (see online documentation²⁰), facilitating the exploration of large design spaces with 100s of constructs feasible with BASIC DNA assembly¹⁰. Meanwhile, the designer interface of the Web App (Figure 2) offers users an intuitive way to create BasicAssembly objects by dragging and dropping selected BasicPart and BasicLinker objects. In addition to visualizing parts using the Web App (Supplementary Figure S2 & S3), users can dynamically visualise assemblies to ensure they contain the desired sequence prior to implementing the checks illustrated in Figure 1c and committing the assembly to the build – a collection of BasicAssembly objects.

BasicBuild Open Standard

Following design, a user builds their collection of assemblies. Multiple calculations are required to determine build instructions for this step¹⁰. Firstly, the unique set of clip reactions required by all assemblies must

be calculated. Each clip reaction is defined by a part in combination with prefix and suffix linker halves. An association between each unique clip reaction and the assemblies requiring it must be made. Secondly, the absolute number of each clip reaction must be determined given each clip reaction can support 15-30 assemblies, depending on the method used. Thirdly, calculations to ensure each part is at a final concentration of 2.5 nM following clip reaction setup must be made to maximize assembly efficiency. These three parameters then guide liquid-handling operations during clip reaction setup and assembly stages of the BASIC workflow. Previously, we implemented this for a specific liquid-handling platform¹⁰ and in this work we describe an adaptable solution for bespoke manual and/or automated workflows.

Using basicsynbio, users can generate BasicBuild objects which contain data including parameters necessary to build collections of BasicAssembly objects. Figure 3 illustrates the four nested objects from an example BasicBuild serialized in JavaScript Object Notation (JSON). The clips_data object contains data on each clip reaction required for the build. Further information on each component is available within unique_parts and unique_linkers objects, where the corresponding key can be used to access this information e.g., “UP0” to access the first part within unique_parts. A link between each clip reaction and the assemblies using it is established by both the “assembly keys” attribute in the clips_data object and the “clips reactions” attribute of the assembly data object. The absolute number of each clip reaction can be calculated using the clips_data “total assemblies” attribute, considering the number of assemblies supplied by each purified clip reaction (15 – 30 depending on the method). To aid the addition of parts to a final concentration of 2.5 nM in clip reactions, a “Part mass for 30 μ L clip reaction (ng)” attribute is provided, where the addition of the associated mass to a 30 μ L clip reaction results in the desired final concentration. To demonstrate the flexibility of this standard, we have written functions that convert BasicBuild objects into manual instructions in pdf format and instructions for a Beckman Echo liquid handling platform.

It is also worth noting that BasicBuilds serialized in JSON can be decoded into Python objects using the API. As such, designs serialized in one location can be transferred securely to the location of manufacturing, decoded, and processed into build instructions specific to the facility. We envision this will allow designers to work

agnostically of the protocol or facility used for building, freeing them to focus on other steps of the Design-Build-Test-Learn cycle.

BASIC SEVA collection

To demonstrate basicsynbio, we aimed to assemble a collection of BASIC DNA assembly compatible vectors based on core SEVA modules and containing a counter-selection cassette. This would make applications requiring multiple plasmids or using specific origins of replication more accessible for BASIC DNA assembly users. Furthermore, adopting the SEVA format and including a counter-selection cassette generates vectors with greater functionality compared to those previously made available⁷.

We designed each vector of the collection to have several functional regions as illustrated in Figure 4a. As with vectors from the SEVA database¹⁵, each BASIC SEVA vector contains a specific combination of antibiotic resistance marker and origin of replication (ori) flanked by SEVA T0 and T1 terminators which prevent transcriptional readthrough from the intervening region, maintaining stability across different designs. The region encompassed by terminators is again flanked by LMP and LMS linkers, enabling it to function as a BasicPart in subsequent assemblies for downstream applications.

The marker and ori are separated by a linker sequence (BSEVA L1) which enables combinatorial assembly of these modules using BASIC. This linker sequence was designed using DNA Chisel²³ to retain maximum plasmid stability. Specifically, sequences that could lead to expression such as promoters and RBSs were avoided and complementarity to the *E. coli* MG1655 genome or existing BASIC parts & linkers was minimized. This latter attribute would reduce the propensity for recombination in strains with active homologous recombination machinery, improving stability²⁴.

The region of the vector outside the LMP/S flanked region is lost during the assembly of downstream constructs using these vectors. We incorporated an mScarlet counter-selection cassette within this region, implying colonies containing the desired assembly will not display a pink phenotype. Any colonies displaying this phenotype will have been transformed by undigested vector and should be avoided. This visual screening feature further increase accuracy and robustness.

To assemble the collection, we split the design in Figure 4a into three BASIC parts: T0 + marker part, ori + T1 part and mScarlet counter-selection cassette. Prior to assembling the collection, we prepped plasmid DNA and sequence verified each part (Materials and methods). We subsequently assembled each vector of the collection *in silico* using basicsynbio (Supplementary Data: addgene_submission.pdf), naming each vector according to the BASIC SEVA nomenclature (Figure 4b). Using the resulting BasicBuild object we exported manual instructions for the entire assembly and Echo liquid-handling instructions for the “Assembly reaction” step of the workflow, aiding building (Materials and methods). Following assembly and transformation, we selected colonies using antibiotics and concentrations given in Figure 4b, picking pink colonies and prepping plasmid DNA. We verified the presence of the correct ori + T1 part in vectors using Sanger sequencing. Sequence data describing the entire collection was exported using basicsynbio and used to generate a basicsynbio PartLinkerCollection making the collection accessible to other basicsynbio users.

The BASIC SEVA collection generated in this work encompasses 30 vectors, every combination of six markers and five oris described in Figure 4b. The six markers correspond with the first six markers used by the SEVA collection. Apart from the tetracycline module (5a), all marker modules are identical in sequence to SEVA modules. For reasons outlined in the Supplementary Information (BASIC SEVA modules) we chose to retain this sequence over that used for plasmids in the SEVA database but note the difference and assign a different string “5a” to distinguish the two. Three of the five oris selected (6, 7 & 9) are identical in sequence to previously described SEVA modules. Meanwhile, ori 5a is homologous to the SEVA RSF1010 module with the exact sequence previously reported in the literature²⁵.

The 5 ori modules used to generate the collection enable a variety of applications. Notably, we include a temperature-sensitive ori (7_pKD46), not present in the SEVA database. This ori is identical in sequence to the ori present in pKD46²⁶. Plasmids harbouring this ori are ideal for applications where plasmid curing is a requirement e.g. strain engineering^{26–28}. The three SEVA oris used (6, 7 & 9) are from three different incompatibility groups²⁹, enabling applications requiring multiple plasmid types in the same host. Furthermore, these three oris further provide a range of copy numbers to tune gene expression. As previously discussed¹⁴, the remaining ori (RSF1010) is compatible with

a broad range of hosts enabling applications suited to non-model organisms. While a high copy number ori was not included in the collection (Supplementary information - BASIC SEVA modules), plasmids containing pBR322 can be amplified with the addition of chloramphenicol²⁹. Additionally, we observed a relatively higher yield for vector BASIC_SEVA_39.10 which contains both pBR322 ori and a chloramphenicol marker (data not shown), suggesting this vector is suitable for applications requiring very high yields of plasmid DNA.

In conclusion, with basicsynbio users can access commonly used parts and linkers, robustly design new parts, linkers, and assemblies and export sequence data and/or build instructions. Notably, the BasicBuild Open Standard is easily parsed into custom build instructions as demonstrated in this work for manual workflows and workflows using acoustic liquid-handlers. To demonstrate basicsynbio we design and assemble a collection of 30 BASIC-DNA-assembly-compatible vectors using modules from the SEVA database. Sequence data for this collection is available for users via the basicsynbio API and plasmids were deposited on Addgene. In combination with other accessible parts and linkers users can easily and robustly design a large repertoire of assemblies enabling applications in Synthetic Biology and the Life Sciences.

Author Contributions (CRediT author statement)

M.C.H.: Conceptualization, Investigation, Software, Visualization, Writing – Original Draft, Writing – Review & Editing, Project Administration

B.C.: Software, Writing – Review & Editing, Visualization

J.M.: Investigation, Writing – Review & Editing

M.S.: Conceptualization, Investigation, Supervision, Project Administration, Writing – Review & Editing

P.F.: Funding Acquisition, Supervision, Writing – Review & Editing.

Acknowledgments

P.F. and M.C.H. are supported by UKRI Engineering and Physical Sciences Research Council (EP/T013788/1). We also thank Alexis Casas for discussion on software and the BasicBuild Open Standard.

Competing interests

P.F. sits on the SAB of Tierra Biosciences.

References

1. Freemont, P. S. Synthetic biology industry: data-driven design is creating new opportunities in biotechnology. *Emerg. Top. life Sci.* **3**, 651–657 (2019).
2. Casini, A., Storch, M., Baldwin, G. S. & Ellis, T. Bricks and blueprints: methods and standards for DNA assembly. *Nat. Rev. Mol. Cell Biol.* **16**, 568–76 (2015).
3. Young, R., Haines, M., Storch, M. & Freemont, P. S. Combinatorial metabolic pathway assembly approaches and toolkits for modular assembly. *Metab. Eng.* **63**, 81–101 (2021).
4. Deb, S. S. & Reshamwala, S. M. S. Recent Progress in DNA Parts Standardization and Characterization. in *Advances in Synthetic Biology* 43–69 (Springer Singapore, 2020). doi:10.1007/978-981-15-0081-7_4.
5. Weber, E., Engler, C., Gruetzner, R., Werner, S. & Marillonnet, S. A modular cloning system for standardized assembly of multigene constructs. *PLoS One* **6**, e16765 (2011).
6. Sarrion-Perdigones, A. *et al.* GoldenBraid: an iterative cloning system for standardized assembly of reusable genetic modules. *PLoS One* **6**, e21622 (2011).
7. Storch, M. *et al.* BASIC: A New Biopart Assembly Standard for Idempotent Cloning Provides Accurate, Single-Tier DNA Assembly for Synthetic Biology. *ACS Synth. Biol.* **4**, 781–7 (2015).
8. Storch, M., Dwijayanti, A., Mallick, H., Haines, M. C. & Baldwin, G. S. BASIC: A Simple and Accurate Modular DNA Assembly Method. *Methods Mol. Biol.* **2205**, 239–253 (2020).
9. Storch, M., Casini, A., Mackrow, B., Ellis, T. & Baldwin, G. S. BASIC: A simple and accurate modular DNA assembly method. in *Methods in Molecular Biology* vol. 1472 79–91 (Humana Press Inc., 2017).
10. Storch, M., Haines, M. C. & Baldwin, G. S. DNA-BOT: a low-cost, automated DNA assembly platform for synthetic biology. *Synth. Biol.* **5**, (2020).
11. Bartasun, P. *et al.* The effect of modulating the quantity of enzymes in a model ethanol pathway on metabolic flux in *Synechocystis* sp. PCC 6803. *PeerJ* **7**, e7529 (2019).
12. Dwijayanti, A., Storch, M., Stan, G. B. & Baldwin, G. S. A modular RNA interference system for gene regulation and robust dynamic feedback control. *bioRxiv* 2019.12.12.873844 (2019) doi:10.1101/2019.12.12.873844.
13. Benns, H. J. *et al.* Prioritization of antimicrobial targets by CRISPR-based oligo recombineering 1 2. *bioRxiv* 2021.02.04.429737 (2021) doi:10.1101/2021.02.04.429737.

14. Silva-Rocha, R. *et al.* The Standard European Vector Architecture (SEVA): a coherent platform for the analysis and deployment of complex prokaryotic phenotypes. *Nucleic Acids Res.* **41**, D666–D675 (2013).
15. Martínez-García, E. *et al.* SEVA 3.0: an update of the Standard European Vector Architecture for enabling portability of genetic constructs among diverse bacterial hosts. *Nucleic Acids Res.* **48**, D1164–D1170 (2020).
16. Casas, A., Bultelle, M., Motraghi, C. & Kitney, R. I. Removing the Bottleneck: Introducing cMatch - a Lightweight Tool for Construct-Matching in Synthetic Biology. *Front. Bioeng. Biotechnol.* **0**, 1409 (1AD).
17. Cock, P. J. A. *et al.* Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics* **25**, 1422–3 (2009).
18. Dwijayanti, A. Engineering standardised and modular biological controllers for efficient design and easy implementation in synthetic genetic circuits. (Imperial College London, 2019). doi:10.25560/82476.
19. Zulkower, V. & Rosser, S. DNA Features Viewer: a sequence annotation formatting and plotting library for Python. *Bioinformatics* **36**, 4350–4352 (2020).
20. basicsynbio documentation. <https://londonbiofoundry.github.io/basicsynbio/>.
21. Untergasser, A. *et al.* Primer3--new capabilities and interfaces. *Nucleic Acids Res.* **40**, e115 (2012).
22. Phillips, D. *Python 3 Object-Oriented Programming (python 3.8)*. vol. 84 (2018).
23. Zulkower, V. & Rosser, S. DNA Chisel, a versatile sequence optimizer. *Bioinformatics* **36**, 4508–4509 (2020).
24. Sleight, S. C., Bartley, B. A., Lieviant, J. A. & Sauro, H. M. Designing and engineering evolutionary robust genetic circuits. *J. Biol. Eng.* **4**, 12 (2010).
25. Cook, T. B. *et al.* Genetic tools for reliable gene expression and recombineering in *Pseudomonas putida*. *J. Ind. Microbiol. Biotechnol.* **45**, 517–527 (2018).
26. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 6640–6645 (2000).
27. Jensen, S. I., Lennen, R. M., Herrgård, M. J. & Nielsen, A. T. Seven gene deletions in seven days: Fast generation of *Escherichia coli* strains tolerant to acetate and osmotic stress. *Sci. Rep.* **5**, 17874 (2016).
28. Vo, P. L. H. *et al.* CRISPR RNA-guided integrases for high-efficiency, multiplexed bacterial genome engineering. *Nat. Biotechnol.* **2020** 1–10 (2020) doi:10.1038/s41587-020-00745-y.
29. Sambrook, J. & Russell, D. *Molecular cloning A Laboratory Manual*. (Cold Spring Harbor Laboratory Press, 2001).

The screenshot displays the 'Standard Part Linker from Collection' interface. At the top, there are dropdown menus for selecting collections: 'BASIC_SEVA_PARTS | v6.1', 'BASIC_BIOLEGIO_LINKERS | v6.1', and 'BASIC_CDS_PARTS | v6.1'. Below these, a search bar is labeled 'Search for Part/Linker' with a dropdown menu showing 'LMS'. A section titled 'Standard Part Linker from Collection' contains a list of parts: 'BASIC_SEVA_18', 'LMP', 'B-P31_Terminator1_LacI_RiboA', 'BASIC_sfGFP_CDS', 'UTR1-RBS3', and 'LMS'. Each part has a small icon and a 'Linker' button. Below the parts list, there are buttons for 'VISUALISE', 'VALIDATE', 'VIEW CURRENT BUILD', and 'ADD TO BUILD'. On the right side, there is a 'Custom Part Linker' section with a 'GENBANK' button and a 'FASTA' button. It includes a checkbox for 'Does the file contains multiple parts / linkers?' and a checkbox for 'Add L_S and L_P sequence to part?'. Below these checkboxes is a large area for uploading a Genbank file, with a prompt 'Drag and drop some files here, or click to select files.' and a 'Draggable Part Linker from file' section. At the bottom right, there are 'BACK' and 'NEXT' buttons.

Figure 2. Screenshot of basicsynbio Web App designer. In this example, BasicParts & BasicLinkers from collections are used to assemble a construct expressing sfGFP under an IPTG-inducible, insulated promoter (B-P31_Terminator1_LacI_RiboA). The assembly is checked against errors and can be visualized prior to adding to build.

```

"unique_parts": {
  "UP0": {
    "sequence": "GGTAAGAACTCGCACTTCGTGGAAACACTATTATCTG...",
    "id": "bb90c9b9713d85ff695493fde3f0051a",
    "name": "BASIC_SEVA_19",
    "description": "BASIC SEVA vector containing Ampic...",
    "part mass per 30 µL clip reaction (ng)": 155,
    "clip reactions": [
      "CR0"
    ]
  },...
}

"unique_linkers": {
  "UL0": {
    "id": "1b62864ef7e8ad38d30e92a2dbc17670",
    "linker_class": "<class 'basicsynbio.main.BasicLin...",
    "sequence": "GGCTCGGGAGACCTATCGGTAATAACAGTCCAATCTG...",
    "prefix_id": "LMS-P",
    "suffix_id": "LMS-S",
    "overhang_slice_params": null,
    "prefix clip reactions": [
      "CR0"
    ],
    "suffix clip reactions": [
      "CR1",
      "CR2",
      "CR3"
    ]
  },...
}

"clips_data": {
  "CR0": {
    "prefix": {
      "key": "UL0",
      "prefix_id": "LMS-P"
    },
    "part": {
      "key": "UP0",
      "id": "bb90c9b9713d85ff695493fde3f0051a",
      "name": "BASIC_SEVA_18"
    },
    "suffix": {
      "key": "UL1",
      "suffix_id": "LMP-S"
    },
    "total assemblies": 3,
    "assembly keys": [
      "A0",
      "A1",
      "A2"
    ]
  },...
}

"assembly_data": {
  "A0": {
    "id": "sfGFP",
    "clip reactions": [
      "CR0",
      "CR1"
    ]
  },...
}

```

Figure 3. BasicBuild Open Standard – Key objects from a BasicBuild example, serialized in JSON: “unique_parts”, “unique_linkers”, “clips_data” and “assembly_data”. For clarity values have been shortened and similar objects replaced with “...”. Using this open standard basicsynbio users can transfer data securely and parse builds into instructions for various workflows. A specification of the BasicBuild Open Standard v0.1 is available online (<https://basicsynbio.web.app/basicbuild-standard>).

Figure 4. BASIC SEVA vector collection – a) Plasmid map of the collection (version 10). BASIC linkers, and SEVA terminators are indicated by magenta and white features, respectively. Origins of replication (yellow) and antibiotic resistance markers (green) are joined via a neutral “BSEVA L1” linker. An mScarlet counter-selection cassette, comprising promoter (blue), RBS (grey) and mScarlet CDS is flanked by methylated LMP and LMS linkers, resulting in drop-out during assembly. SEVA-T0-rev & SEVA-T1-for sequencing primer binding sites are indicated. b) Nomenclature of the BASIC SEVA collection. Vectors are named through the combination of two strings that reflect the origin of replication and resistance marker identities. Where an integer value is given the associated module is identical to that provided by SEVA¹⁵. The remaining modules are discussed in the text. The nomenclature also provides a versioning system, equivalent to that used by GenBank (<https://www.ncbi.nlm.nih.gov/genbank/release/>). Antibiotic concentrations used to isolate plasmids in this study are given (Carbenicillin was used instead of Ampicillin¹ and only spectinomycin was tested²).