

Supporting Information

DNA-BOT: A low-cost, automated DNA assembly platform for synthetic biology, Storch et al. 2019

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Materials and methods

BASIC DNA parts and availability

DNA parts from Table S1 with exception of BASIC_SEVA_37_CmR-p15A_v1.0 were cloned into a Bsal-site-free, Amp-pUC storage vector. The resulting plasmids and BASIC_SEVA_37_CmR-p15A_v1.0 were prepared at midiprep scale using GenScript®'s Plasmid DNA Prep Service and diluted to 200 ng/µL, ready to use in clip reactions.

A control plasmid containing only the backbone was derived from BASIC_SEVA_37_CmR-p15A_v1.0: Bsal digested plasmid was enzymatically blunted, self-ligated, transformed in DH5alpha and a correct clone identified via Sanger sequencing.

All DNA parts are available upon request.

DNA-BOT

A csv file describing each of the 88 constructs was generated along with csv files describing the Biolegio BASIC linker set (BBP-19100) standard linkers and the BASIC DNA parts required^{1,2}. As described in DNA-BOT_instructions_v1.0.0 (Supporting Information), these csv files were used to generate four Opentrons OT-2 scripts. The 4th script was modified to transform and spot control plasmid and no plasmid controls in wells A12 – H12. Furthermore, a 5th script was generated separately to spot 10 µL of each transformation reaction. These scripts were implemented as described in DNA-BOT_instructions_v1.0.0 using LB-agar plates supplemented with 25 µg/mL chloramphenicol. All scripts are available at <https://github.com/BASIC-DNA-ASSEMBLY/dnabot>.

Flow cytometry

Individual colonies were picked from agar plates generated by DNA-BOT and 200 µL LB medium supplemented with 25 µg/mL chloramphenicol inoculated in 96-well plates. Cultures were incubated overnight, shaking 600 rpm at 30°C. Overnight cultures were diluted 200 times into 100 µL LB supplemented with 25 µg/mL chloramphenicol. Cultures were grown shaking at 30°C for 6 hours and 2 µL off-sampled into 200 µL Phosphate Buffer Saline supplemented with 2 mg/mL kanamycin. Samples were analysed for GFP, BFP and RFP fluorescence using a BD Fortessa flow cytometer with all samples gated using the same forward and side scatter settings. Data was analysed using FlowJo_V10 and subsequently processed as described in the main text.

Supplementary figures

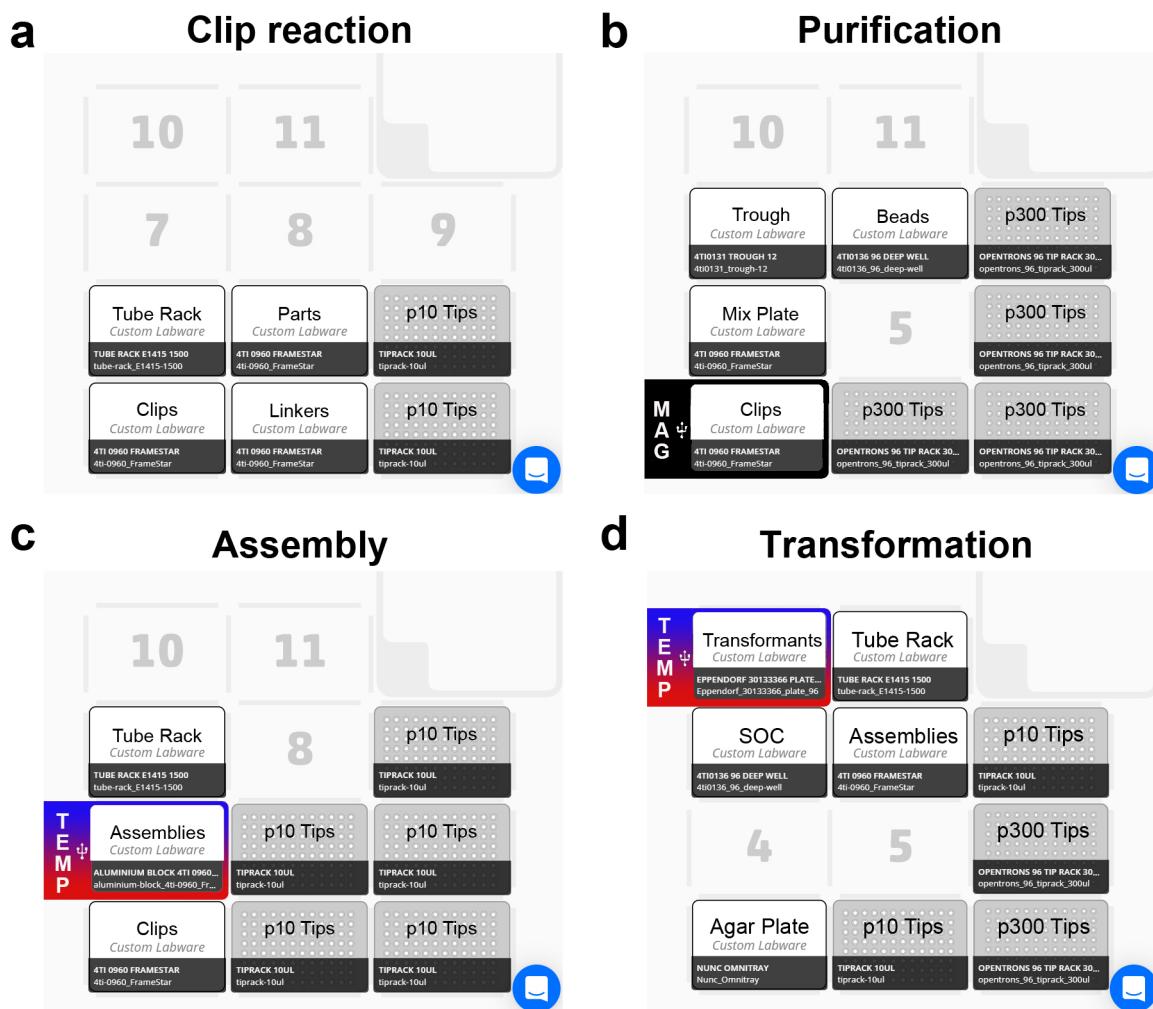


Figure S1. Deck layout used during generation of 88 constructs using DNA BOT (a) Script 1: Clip reaction, (b) Script 2: Purification, (c) Script 3: Assembly and (d) Script 4: Transformation.

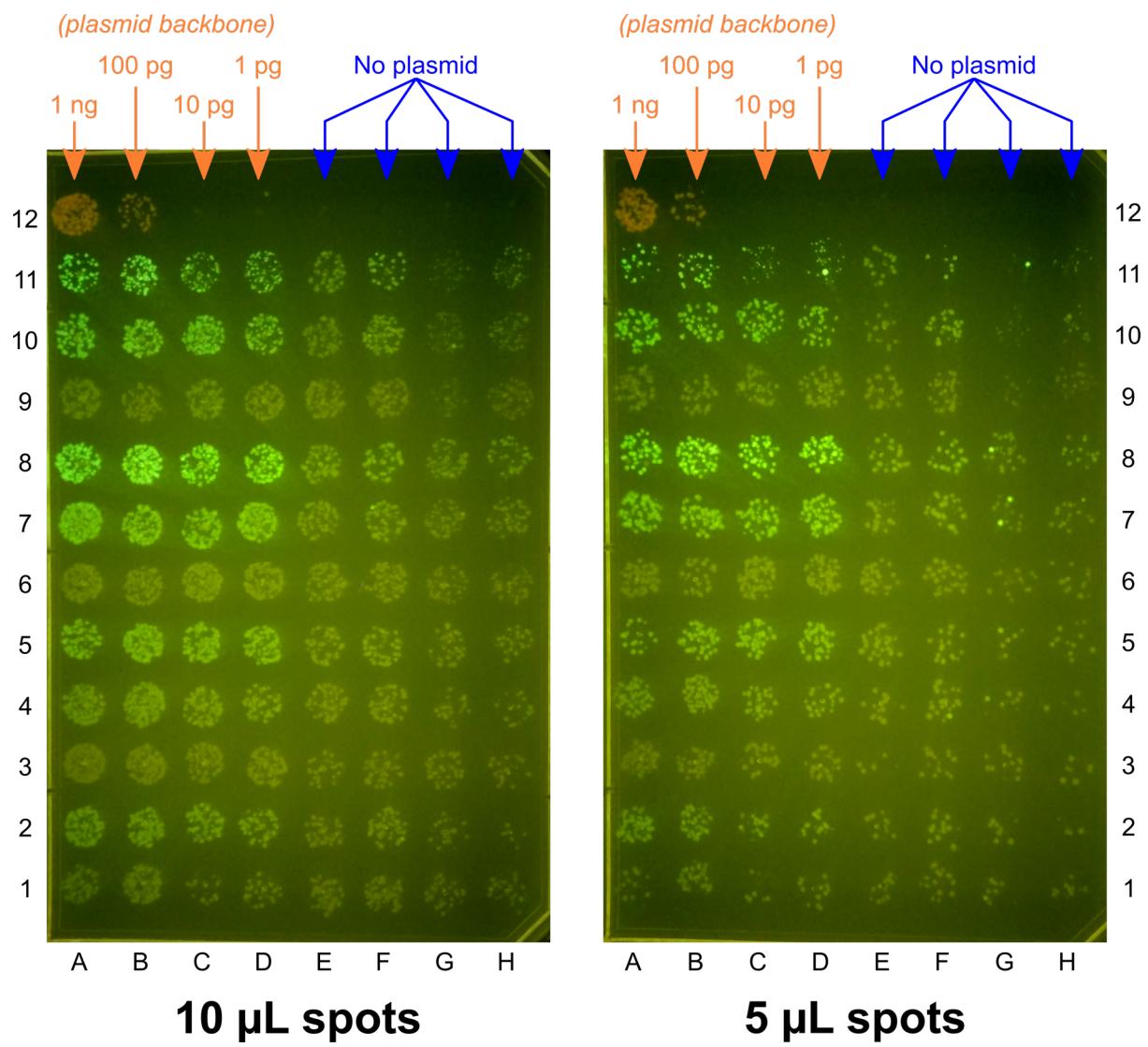


Figure S2. Agar plates imaged for GFP on a Safe Imager™ 2.0 Blue Light Transilluminator. Plates were spotted with 10 or 5 μ L of each transformation reaction. Corresponding well identities can be inferred by the surrounding grid of letters and numbers. Cells spotted on positions A12 – H12, were transformed with 1 ng, 100 pg, 10 pg, 1 pg of BASIC_SEVA_37_CmR-p15A_v1.0 (plasmid backbone with mScarlet counter selection cassette) or with plasmid-free H₂O (no plasmid), respectively. (Materials and methods).

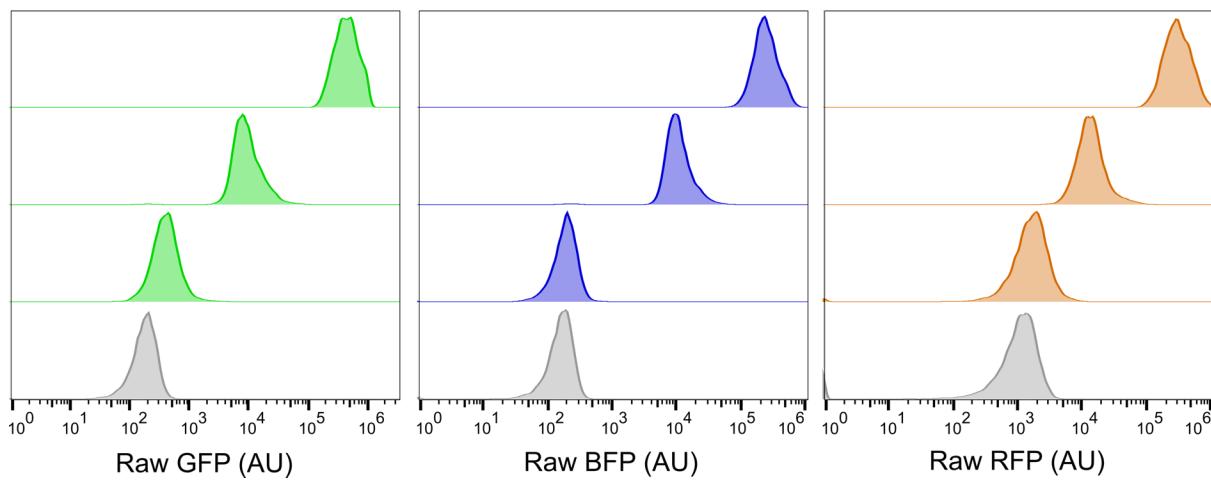


Figure S3. Flow cytometry histograms illustrating dynamic range in fluorescent reporter expression strength. Negative control cells with empty backbone are shown in grey and examples for low, medium and high expression phenotypes from the 88 construct library are shown in green (GFP), blue (BFP) and orange (RFP). Specifically, data for the following constructs are shown in order of low, medium and high expression, respectively: GFP: G3, H5 and C11. BFP expression: E2, H1 and H10. RFP: A1, F4 and H10.

Supplementary tables

Table S1: DNA Sequences for BASIC parts used in this work

BASIC prefix (`TCTGGTGGGTCTCTGTCC`) and suffix (`GGCTCGGGAGACCTATCG`) sequences, along with the mScarlet coding sequence (`magenta`) are highlighted.

ID	SynBioHub URI
BASIC_L3S2P21_J23105_RiboJ	<code>TCTGGTGGGTCTCTGTCC</code> CTCGTACCAAATTCCAGAAAAGAGGCCTCCGAAAGGGGGCCTTTTCGT TTGGTCCGTGCCTACTCTGGAAAATCTTACCGCTAGCTAGCCTAGGTACTATGCTAGCAGCTGCA CCGGATGTGCTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATAATTGTTAA <code>GGCT</code> <code>CGGGAGACCTATCG</code>
BASIC_L3S2P21_J23106_RiboJ	<code>TCTGGTGGGTCTCTGTCC</code> CTCGTACCAAATTCCAGAAAAGAGGCCTCCGAAAGGGGGCCTTTTCGT TTGGTCCGTGCCTACTCTGGAAAATCTTACCGCTAGCTAGCCTAGGTATGTCTAGCAGCTGCA CCGGATGTGCTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATAATTGTTAA <code>GGCT</code> <code>CGGGAGACCTATCG</code>
BASIC_L3S2P21_J23101_RiboJ	<code>TCTGGTGGGTCTCTGTCC</code> CTCGTACCAAATTCCAGAAAAGAGGCCTCCGAAAGGGGGCCTTTTCGT TTGGTCCGTGCCTACTCTGGAAAATCTTACAGCTAGCTAGCCTAGGTATTGCTAGCAGCTGCA CCGGATGTGCTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATAATTGTTAA <code>GGCT</code> <code>CGGGAGACCTATCG</code>
BASIC_L3S2P21_J23104_RiboJ	<code>TCTGGTGGGTCTCTGTCC</code> CTCGTACCAAATTCCAGAAAAGAGGCCTCCGAAAGGGGGCCTTTTCGT TTGGTCCGTGCCTACTCTGGAAAATCTTACAGCTAGCTAGCCTAGGTATTGCTAGCAGCTGCA CCGGATGTGCTTCCGGTCTGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATAATTGTTAA <code>GGCT</code> <code>CGGGAGACCTATCG</code>
BASIC_sfGFP_O RF_v1.0	<code>TCTGGTGGGTCTCTGTCC</code> ATGCGTAAAGGCGAAGAAC TGTTCACGGCGTAGTTCGATCTGGTCGAGCT GGACGGCGATGTGAACGGTCATAAGTTAGCGTTCGCGTGAGGTGAGGGCGACCGAACGGAAAC TGACCCCTGAAGTTCATCTGCACCACCGTAAACTGCCGTGCTGGCCGACCTGGTGACGACGTTGACG TATGGCGTCGAGTGTGCGTGTATCCGGACACATGAAACAACACGATTCTCAAATCTGCATGCC GGAGGGTTACGTCAGGAGCGTACCATTCCTCAAGGATGATGGCACTTACAAACTCGCGAGAGGTTA AGTTGAAGGTGACACGCTGGTCAATGTTGAAAGGTATGACTTAAAGAGGATGGTAACATT CTGGGCCATAAACTGGAGTATAACTCAACAGCCATAATGTTACATTACGGCAGACAAGCAAAGCAGG CATCAAGGCCAATTCAAGATTGCCACAATGTTGAGGACGGTAGCGTCCAACGGGACATTACAGC AGAACACCCCAATTGGTGACGGTCCGGTTGCTGCCGATAATCACTATGAGCACCCAAAGCGTGTG AGCAAAGATCCGAACGAAAAACGTGATCACATGGCCTGCTGGAATTGTAACGGCTGGGCATACCCA CGGTATGGACGAGCTGTATAAGCGTCCGTA <code>GGCTCGGGAGACCTATCG</code>
BASIC_RFP_OR F_v1.0 (mCherry)	<code>TCTGGTGGGTCTCTGTCC</code> ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTT CATGCG CTTCAAGGTGCACATGGAGGGCTCCGTGAACGCCACGGAGTTGAGATCGAGGGCGAGGGCGAGGGCGCC CCTACGAGGGCACCCAGGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTGCCGGACATC CTGTCCTCAGTTCATGTCAGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAA GCTGTCCTCCCCGAGGGCTCAAGTGGAGCCGTGATGAACTCGAGGACGGCGCGTGGTGACCGTGA CCCAGGACTCCTCCTGCAAGGACGCGAGTTCATACAAGGTGAAGCTGCCGGACCAACTCCCTCC GACGGCCCGTAATGCAAGAAGACCATGGCTGGAGGCCCTCCGAGCGGATGTACCCCGAGGACGG CGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGA CCACCTACAAGGCCAAGAACGCCGTGCAAGCTGCCGGCGCTACAACGTCAACATCAAGTTGGACATCACC TCCCACAACGAGGACTACACCATCGTGAACAGTACGAACGCCGGAGGGCGCCACTCCACCGCGGCAT GGACGAGCTGTACAAGTAA <code>GGCTCGGGAGACCTATCG</code>
BASIC_BFP_OR F_v1.0	<code>TCTGGTGGGTCTCTGTCC</code> ATGTCCGAGTTGATCAAAGAGAACATGCATATGAAATTATATGGAAGGCAC TGTAGATAATCATCATTAAATGTAACGTCGGAAGGCAGAGGTAACCATATGAGGTACGCA GCATCAAGGTGGTGGAGGGCGTCCGCTGCCATTGCTTGGATATTGACGAGCTTCCTCTACGGT TCTAAAACTTCATCAATCACACGCAAGGTATTCCGGACTTCTTAAACAGTCGTTCCGGAGGGTTCAAC

Table S2: DNA Sequences for BASIC linkers used in this work

UTR-RBS linkers were used to build the operons and methylated linkers LMP & LMS were used to connect the expression cassette with the backbone. The 3 Biobrick RBS sequences (underlined) encoded on the UTR-RBS linkers were in order from low to high translation strength: BBa_B0033 (RBS1), BBa_B0064 (RBS2), BBa_B0034 (RBS3). Central uppercase sequence sections indicate the homology regions of the single stranded 21 base overhangs, which bring Clips together during final assembly.

UTR1-RBS1	ctcggtgaacaccgtcTCAGGTAAGTATCAGTTGTAA <u>atcacacaggact</u> agtcc
UTR1-RBS2	ctcggtgaacaccgtcTCAGGTAAGTATCAGTTGTAA <u>aaagaggggaaat</u> agtcc
UTR1-RBS3	ctcggtgaacaccgtcTCAGGTAAGTATCAGTTGTAA <u>aaagaggagaaat</u> agtcc
UTR2-RBS1	ctcggttactattggCTGAGATAAGGGTAGCAGAAA <u>atcacacaggact</u> agtcc
UTR2-RBS3	ctcggttactattggCTGAGATAAGGGTAGCAGAAA <u>aaagaggagaaat</u> agtcc
UTR3-RBS1	ctcggttatctcggtCTGACGGTAAAATCTATTGT <u>atcacacaggact</u> agtcc
UTR3-RBS3	ctcggttatctcggtCTGACGGTAAAATCTATTGT <u>aaagaggagaaat</u> agtcc
LMP	ctcggttaaga <u>actcg</u> CACTCGTGGAAACACTATT <u>atctggtgggtctgtcc</u>
LMS	ctcggtgag <u>acccatcg</u> GTAATAACAGTCCAATCTGGTG <u>taacttcggaatcg</u> tcc

Table S3: DNA-BOT running costs per construct

Costing assumes constructs are composed of 5 parts and each purified clip is used in 15 assemblies, representative for the 88 constructs. Costs for foil seals, linkers, trough, microcentrifuge tubes, assembly buffer, ethanol and water were considered neglectable. Unit prices correct as of October 2019 and values give to 2 DP.

Item	Units	Units per construct	Unit price (USD)	Cost (USD)
Opentrons p10 tips	tips	9.33	0.03	0.27
Framestar 96-well Rigid and Skirted PCR Plates	plates	0.02	3.69	0.06
Promega T4 DNA Ligase	µL	0.17	1.18	0.20
NEB BsAl-HF®v2	µL	0.33	1.11	0.37
Opentrons p300 tips	tips	5.00	0.03	0.14
Brooks Life Sciences 96 Square Deep Well Storage Microplate	plates	0.01	3.69	0.04
Beckman Coulter™ Agencourt AMPure XP SPRI paramagnetic beads	mL	0.02	16.62	0.30
NEB® 5-alpha Competent <i>E.</i> <i>coli</i> , 96 well plate	plate	0.01	380.12	3.96
Thermo Scientific™ Nunc™ OmniTray™ Single-Well Plate	plate	0.01	3.58	0.04
Total				5.37
Total – w/o cells				1.41

Table S4: Hands-on time required for manual BASIC assembly and DNA-BOT.

Calculated for the implementation of 48 clip reactions, plus the assembly and transformation of 88 constructs. The Q_{time} metric³ has been calculated from these two values.

Step	Manual protocol (mins)	Automated protocol (mins)
Clip reactions (48)	60	15
Purification	30	25
Assembly	90	15
Transformation	100	35
Total	280	90
$Q_{time} = 3.11$		

Bibliography

- (1) Biolegio BASIC linkers <https://www.biolegio.com/products-services/basic/>.
- (2) BASIC Assembly website www.basic-assembly.org.
- (3) Walsh, D. I.; Pavan, M.; Ortiz, L.; Wick, S.; Bobrow, J.; Guido, N. J.; Leinicke, S.; Fu, D.; Pandit, S.; Qin, L.; et al. Standardizing Automated DNA Assembly: Best Practices, Metrics, and Protocols Using Robots. *SLAS Technol. Transl. Life Sci. Innov.* **2019**, 247263031882533. <https://doi.org/10.1177/2472630318825335>.