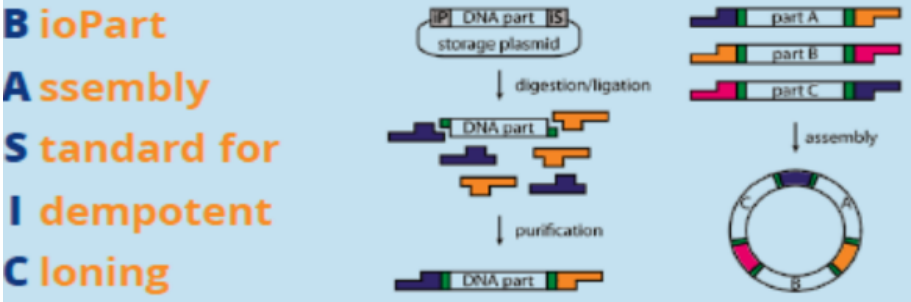
Protocol for BasicBuild 25-09-2021



Materials

Item	Order number (if applicable)
PCR machine	
Water bath (42°C) for transformation	
Magnetic plate	Ambion AM10050 (Thermo)
Benchtop centrifuge	
Microplate centrifuge	
Vortex	
96 well U-Bottom Falcon plate	Falcon 351177 (Thermo)
Microcentrifuge tubes	
PCR tubes	
Brooks Life Sciences PCR Foil Seals	4ti-0550
Pipettes/tips 10 and 200 µl	
Agencourt AMPure XP SPRI paramagnetic beads	A6388 (Beckman Coulter)
ddH20	
70% EtOH	
Biolegio BASIC linkers	
NEB BsaI-HF v2 enzyme (R3733) 20 U/µl	R3733 (NEB)
Promega T4 ligase (M1801) 1-3U/µl	M1801 (Promega)
[optional] 10x Assembly Buffer: 0.2 M Tris:HCl (pH 8.0), 0.1 M MgCl2, 0.5 M KCl	
Chemically competent cells (DH5alpha, 1x109 CFU/µg pUC19	C2987I (NEB) or equivalent
SOC media	
Petri dishes	
LB-Agar + antibiotic/s	

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Method

Preperation of BASIC linkers and parts

BASIC linkers can be ordered from Biolegio (info@biolegio.com) and will be delivered in a lyophilized format along with linker annealing buffer.

1. Spin down plates containing lyophilized linkers.
2. Add 150 µl of linker annealing buffer to each well, seal the plate with a PCR foil and incubate for 1 hour at room temperate.
3. Vortex the plate and collect liquid via centrifugation.
4. Conduct the following incubation in a thermocycler:

Temperature (°C)	Time	
95	2 min	
94 (-1 °C/cycle)	40 seconds	x70 cycles
4	Hold	

5. Collect the liquid in wells via centrifugation.
6. Linkers are ready to use or can be stored at -20°C until required.

Clips Reaction

Below contains a table with each clip needed for the assemblies of the build.

Clip Index	Prefix ID	Part Name	Part suggested stock concentration (ng/µL)	Part stock per 30 µL clip (µL)	Suffix ID	Total assemblies	Assembly indexes	Clip plate mapping

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1	LMS-P	BASIC_SEVA_26	141	1	LMP-S	63	[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63]	nan
2	LMP-P	B-P1_Terminator1_J23119_RiboA	121	1	UTR1-S	1	[1]	nan

3	UTR1-RBS2-P	BASIC_sfGFP_CDS	146	1	LMS-S	63	[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63]	nan
4	LMP-P	B-P2_Terminator1_J23111_RiboA	121	1	UTR1-S	1	[2]	nan
5	LMP-P	B-P3_Terminator1_J23104_RiboA	121	1	UTR1-S	1	[3]	nan
6	LMP-P	B-P4_Terminator1_J23101_RiboA	121	1	UTR1-S	1	[4]	nan
7	LMP-P	B-P5_Terminator1_J23108_RiboA	121	1	UTR1-S	1	[5]	nan
8	LMP-P	B-P6_Terminator1_J23106_RiboA	121	1	UTR1-S	1	[6]	nan
9	LMP-P	B-P7_Terminator1_J23105_RiboA	121	1	UTR1-S	1	[7]	nan
10	LMP-P	B-P8_Terminator1_J23116_RiboA	121	1	UTR1-S	1	[8]	nan

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11	LMP-P	B-P9_Terminator2_J23119_RiboB	121	1	UTR1-S	1	[9]	nan
12	LMP-P	B-P10_Terminator2_J23111_RiboB	121	1	UTR1-S	1	[10]	nan
13	LMP-P	B-P11_Terminator2_J23104_RiboB	121	1	UTR1-S	1	[11]	nan
14	LMP-P	B-P12_Terminator2_J23101_RiboB	121	1	UTR1-S	1	[12]	nan
15	LMP-P	B-P13_Terminator2_J23108_RiboB	121	1	UTR1-S	1	[13]	nan
16	LMP-P	B-P14_Terminator2_J23106_RiboB	121	1	UTR1-S	1	[14]	nan
17	LMP-P	B-P15_Terminator2_J23105_RiboB	121	1	UTR1-S	1	[15]	nan
18	LMP-P	B-P16_Terminator2_J23116_RiboB	121	1	UTR1-S	1	[16]	nan
19	LMP-P	B-P17_Terminator3_J23119_RiboC	120	1	UTR1-S	1	[17]	nan
20	LMP-P	B-P18_Terminator3_J23111_RiboC	120	1	UTR1-S	1	[18]	nan
21	LMP-P	B-P19_Terminator3_J23104_RiboC	120	1	UTR1-S	1	[19]	nan
22	LMP-P	B-P20_Terminator3_J23101_RiboC	120	1	UTR1-S	1	[20]	nan
23	LMP-P	B-P21_Terminator3_J23108_RiboC	120	1	UTR1-S	1	[21]	nan
24	LMP-P	B-P22_Terminator3_J23106_RiboC	120	1	UTR1-S	1	[22]	nan
25	LMP-P	B-P23_Terminator3_J23105_RiboC	120	1	UTR1-S	1	[23]	nan
26	LMP-P	B-P24_Terminator3_J23116_RiboC	120	1	UTR1-S	1	[24]	nan

27	LMP-P	B-P25_Terminator1_Phlf_RiboA	122	1	UTR1-S	1	[25]	nan
28	LMP-P	B-P26_Terminator1_CymR_RiboA	123	1	UTR1-S	1	[26]	nan
29	LMP-P	B-P27_Terminator1_TetR_RiboA	123	1	UTR1-S	1	[27]	nan
30	LMP-P	B-P28_Terminator1_VanR_RiboA	122	1	UTR1-S	1	[28]	nan
31	LMP-P	B-P29_Terminator1_LuxR_RiboA	122	1	UTR1-S	1	[29]	nan
32	LMP-P	B-P30_Terminator1_CinR_RiboA	130	1	UTR1-S	1	[30]	nan
33	LMP-P	B-P31_Terminator1_LacI_RiboA	122	1	UTR1-S	1	[31]	nan
34	LMP-P	B-P32_Terminator1_AraC_RiboA	133	1	UTR1-S	1	[32]	nan
35	LMP-P	B-P33_Terminator2_Phlf_RiboB	122	1	UTR1-S	1	[33]	nan
36	LMP-P	B-P34_Terminator2_CymR_RiboB	123	1	UTR1-S	1	[34]	nan
37	LMP-P	B-P35_Terminator2_TetR_RiboB	123	1	UTR1-S	1	[35]	nan
38	LMP-P	B-P36_Terminator2_VanR_RiboB	121	1	UTR1-S	1	[36]	nan
39	LMP-P	B-P37_Terminator2_LuxR_RiboB	122	1	UTR1-S	1	[37]	nan
40	LMP-P	B-P38_Terminator2_CinR_RiboB	130	1	UTR1-S	1	[38]	nan
41	LMP-P	B-P39_Terminator2_LacI_RiboB	122	1	UTR1-S	1	[39]	nan
42	LMP-P	B-P40_Terminator2_AraC_RiboB	132	1	UTR1-S	1	[40]	nan
43	LMP-P	B-P41_Terminator3_Phlf_RiboC	122	1	UTR1-S	1	[41]	nan

44	LMP-P	B-P42_Terminator3_CymR_RiboC	123	1	UTR1-S	1	[42]	nan
45	LMP-P	B-P43_Terminator3_TetR_RiboC	122	1	UTR1-S	1	[43]	nan
46	LMP-P	B-P44_Terminator3_VanR_RiboC	121	1	UTR1-S	1	[44]	nan
47	LMP-P	B-P45_Terminator3_LuxR_RiboC	121	1	UTR1-S	1	[45]	nan
48	LMP-P	B-P46_Terminator3_CinR_RiboC	129	1	UTR1-S	1	[46]	nan
49	LMP-P	B-P47_Terminator3_LacI_RiboC	121	1	UTR1-S	1	[47]	nan
50	LMP-P	B-P48_Terminator3_AraC_RiboC	132	1	UTR1-S	1	[48]	nan
51	LMP-P	B-P49_Terminator1_T7_100_RiboA	120	1	UTR1-S	1	[49]	nan
52	LMP-P	B-P50_Terminator1_T7_52_RiboA	120	1	UTR1-S	1	[50]	nan
53	LMP-P	B-P51_Terminator1_T7_50_RiboA	120	1	UTR1-S	1	[51]	nan
54	LMP-P	B-P52_Terminator1_T7_25_RiboA	120	1	UTR1-S	1	[52]	nan
55	LMP-P	B-P53_Terminator2_T7_100_RiboB	120	1	UTR1-S	1	[53]	nan
56	LMP-P	B-P54_Terminator2_T7_52_RiboB	120	1	UTR1-S	1	[54]	nan
57	LMP-P	B-P55_Terminator2_T7_50_RiboB	120	1	UTR1-S	1	[55]	nan
58	LMP-P	B-P56_Terminator2_T7_25_RiboB	120	1	UTR1-S	1	[56]	nan
59	LMP-P	B-P57_Terminator3_T7_100_RiboC	120	1	UTR1-S	1	[57]	nan

60	LMP-P	B-P58_Terminator3_T7_52_RiboC	120	1	UTR1-S	1	[58]	nan
61	LMP-P	B-P59_Terminator3_T7_50_RiboC	120	1	UTR1-S	1	[59]	nan
62	LMP-P	B-P60_Terminator3_T7_25_RiboC	120	1	UTR1-S	1	[60]	nan
63	LMP-P	B-P61_Terminator1_BetI_RiboA	122	1	UTR1-S	1	[61]	nan
64	LMP-P	B-P62_Terminator2_Ttg_RiboB	121	1	UTR1-S	1	[62]	nan
65	LMP-P	B-P63_Terminator3_SalTTC_RiboC	124	1	UTR1-S	1	[63]	nan

Prepare a Master mix for clip reactions, the below table provides the required components for the master mix with sufficient quantities for all clip reactions.

Component	Volume per clip (µL)
Promega T4 DNA Ligase 10x Buffer	219.0
Water	1131.5
NEB BsaI-HFv2	73.0
Promega T4 DNA Ligase	36.5

For each Clip reaction, setup 1 PCR tube with 30 µl total volume:

Dispense 20 uL master mix, 1 µl of each prefix and suffix Linker, 1 µl of part (or more depending on concentration) into a PCR tube and make up to 30 µl with water.

After mixing, tubes are placed in a PCR machine running the following programme:

Temperature (°C)	Time	
37	2 min	
20	1 min	x20 cycles
60	5 min	

Magbead purification

Prepare fresh 70% EtOH (0.5 ml per BASIC reaction) and bring magnetic beads (AmpureXP or Ampliclean) stored at 4°C back into homogeneous mix by shaking thoroughly.

1. Add 54 µl of magnetic beads into 96 well Falcon plate (one well per BASIC reaction) and add the 30 µl BASIC linker ligation from the PCR machine step, mix by pipetting 10 times.
2. Wait 5 min to allow DNA binding to magbeads.
3. Place Falcon plate on magnetic stand and wait for rings to form and solution to clear.
4. Aspirate most of the solution with a 200 µL pipette set to 80 µL.

5 Add 190 µl 70% EtOH to each well and wait 30 s.

6 Remove solution from each well (pipette set to 200 µl volume)

7 Add 190 µl 70% EtOH to each well and wait 30 s.

8 Remove solution from each well (pipette set to 200 µl volume)

9 Leave the plate to dry for 1-2 min.

10 Remove Falcon plate from magnet and resuspend magbeads in 32 µl dH20.

11 Wait 1 min for DNA to elute.

12 Place Falcon plate back on magnetic stand and allow ring to form and solution to clear.

13 Transfer 30 µl of purified clip reaction into a clean microcentrifuge tube or well and store at at -20°C for up to 1 month.

Assembly reaction

Below contains a table with each BASIC assembly within your build

Assembly Index	Assembly ID	Clip indexes	Assembly plate mapping
1	promoter_construct_0	[1, 2, 3]	nan
2	promoter_construct_1	[1, 4, 3]	nan
3	promoter_construct_2	[1, 5, 3]	nan
4	promoter_construct_3	[1, 6, 3]	nan
5	promoter_construct_4	[1, 7, 3]	nan
6	promoter_construct_5	[1, 8, 3]	nan
7	promoter_construct_6	[1, 9, 3]	nan
8	promoter_construct_7	[1, 10, 3]	nan
9	promoter_construct_8	[1, 11, 3]	nan
10	promoter_construct_9	[1, 12, 3]	nan
11	promoter_construct_10	[1, 13, 3]	nan
12	promoter_construct_11	[1, 14, 3]	nan
13	promoter_construct_12	[1, 15, 3]	nan
14	promoter_construct_13	[1, 16, 3]	nan
15	promoter_construct_14	[1, 17, 3]	nan
16	promoter_construct_15	[1, 18, 3]	nan
17	promoter_construct_16	[1, 19, 3]	nan
18	promoter_construct_17	[1, 20, 3]	nan
19	promoter_construct_18	[1, 21, 3]	nan
20	promoter_construct_19	[1, 22, 3]	nan
21	promoter_construct_20	[1, 23, 3]	nan
22	promoter_construct_21	[1, 24, 3]	nan
23	promoter_construct_22	[1, 25, 3]	nan
24	promoter_construct_23	[1, 26, 3]	nan

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25	promoter_construct_24	[1, 27, 3]	nan
26	promoter_construct_25	[1, 28, 3]	nan
27	promoter_construct_26	[1, 29, 3]	nan
28	promoter_construct_27	[1, 30, 3]	nan
29	promoter_construct_28	[1, 31, 3]	nan
30	promoter_construct_29	[1, 32, 3]	nan
31	promoter_construct_30	[1, 33, 3]	nan
32	promoter_construct_31	[1, 34, 3]	nan
33	promoter_construct_32	[1, 35, 3]	nan
34	promoter_construct_33	[1, 36, 3]	nan
35	promoter_construct_34	[1, 37, 3]	nan
36	promoter_construct_35	[1, 38, 3]	nan
37	promoter_construct_36	[1, 39, 3]	nan
38	promoter_construct_37	[1, 40, 3]	nan
39	promoter_construct_38	[1, 41, 3]	nan
40	promoter_construct_39	[1, 42, 3]	nan
41	promoter_construct_40	[1, 43, 3]	nan
42	promoter_construct_41	[1, 44, 3]	nan
43	promoter_construct_42	[1, 45, 3]	nan
44	promoter_construct_43	[1, 46, 3]	nan
45	promoter_construct_44	[1, 47, 3]	nan
46	promoter_construct_45	[1, 48, 3]	nan
47	promoter_construct_46	[1, 49, 3]	nan
48	promoter_construct_47	[1, 50, 3]	nan
49	promoter_construct_48	[1, 51, 3]	nan
50	promoter_construct_49	[1, 52, 3]	nan
51	promoter_construct_50	[1, 53, 3]	nan
52	promoter_construct_51	[1, 54, 3]	nan
53	promoter_construct_52	[1, 55, 3]	nan
54	promoter_construct_53	[1, 56, 3]	nan
55	promoter_construct_54	[1, 57, 3]	nan
56	promoter_construct_55	[1, 58, 3]	nan
57	promoter_construct_56	[1, 59, 3]	nan
58	promoter_construct_57	[1, 60, 3]	nan
59	promoter_construct_58	[1, 61, 3]	nan
60	promoter_construct_59	[1, 62, 3]	nan
61	promoter_construct_60	[1, 63, 3]	nan
62	promoter_construct_61	[1, 64, 3]	nan

63	promoter_construct_62	[1, 65, 3]	nan

For each BASIC assembly, combine the required purified clip reactions in a final volume of 10 µl in 1x Assembly or NEB CutSmart buffer as follows:

Reagent	Volume
10x Assembly Buffer (or 10x NEB CutSmart)	1 µl
Each purified clip reaction required for the assembly	1 µl for each
dH20	Top up to 10 µl total volume

Run assembly reaction in PCR machine with following programme

Temperature (°C)	Time
50	45 min
4	Hold

Transformation

Use 50 µl of chemically competent cells DH5alpha with high transformation efficiency (109 CFU/µg pUC19, for instance NEB C2987I) to transform 5 µl of each BASIC assembly:

1. Chemically competent cells are stored at -80°C.
2. Thaw competent cells on ice (takes 5-10 min); 50 µl per BASIC assembly to be transformed.
3. Cool 5 µl of BASIC DNA assembly in 1.5 ml microcentrifuge tube on ice.
4. Add 50 µl of competent cells to each precooled 5 µl BASIC reaction.
5. Incubate on ice for 20 min.
6. Apply heat shock in 42°C water bath for 45s and place back on ice for 2 min.
7. Add 200 µl SOC medium to each tube and incubate shaking at 37°C for 1h recovery.
8. Spot or plate cells on agar plates with appropriate antibiotics. Depending on number of parts assembled and transformation efficiency 2-250 µl might be spotted or plated.
9. Incubate agar plates at 37°C overnight, next day pick colony for assay or miniprep.