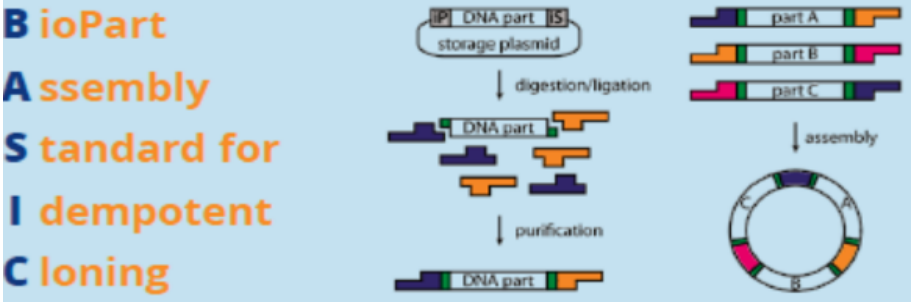
Protocol for BasicBuild 26-08-2021



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	

Protocol for BasicBuild 26-08-2021

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
1	LMP-P	BS_1xx	57	1	BSEVA_L1-S	6	[1, 2, 3, 4, 5, 6]	nan
2	BSEVA_L1-P	BS_x5x	237	1	LMS-S	6	[1, 7, 13, 19, 25, 31]	nan
3	LMS-P	B407_J23119-RBS34-mScarl	37	1	LMP-S	18	[1, 2, 6, 7, 8, 12, 13, 14, 18, 19, 20, 24, 25, 26, 30, 31, 32, 36]	nan
4	BSEVA_L1-P	BS_x6x	100	1	LMS-S	6	[2, 8, 14, 20, 26, 32]	nan
5	BSEVA_L1-P	BS_x7x	134	1	LMS-S	6	[3, 9, 15, 21, 27, 33]	nan

Protocol for BasicBuild 26-08-2021

6	LMS-P	B408_J23119-RBS-A12-mSc	46	1	LMP-S	12	[3, 4, 9, 10, 15, 16, 21, 22, 27, 28, 33, 34]	nan
7	BSEVA_L1-P	BS_x7x_TS_CASc	79	1	LMS-S	6	[4, 10, 16, 22, 28, 34]	nan
8	BSEVA_L1-P	BS_x8x	110	1	LMS-S	6	[5, 11, 17, 23, 29, 35]	nan
9	LMS-P	B405_J23106-RBS34-mScarI	37	1	LMP-S	6	[5, 11, 17, 23, 29, 35]	nan
10	BSEVA_L1-P	BS_x9x	131	1	LMS-S	6	[6, 12, 18, 24, 30, 36]	nan
11	LMP-P	BS_2xx	51	1	BSEVA_L1-S	6	[7, 8, 9, 10, 11, 12]	nan
12	LMP-P	BS_3xx	45	1	BSEVA_L1-S	6	[13, 14, 15, 16, 17, 18]	nan
13	LMP-P	BS_4xx	55	1	BSEVA_L1-S	6	[19, 20, 21, 22, 23, 24]	nan
14	LMP-P	BS_5xx	67	1	BSEVA_L1-S	6	[25, 26, 27, 28, 29, 30]	nan
15	LMP-P	BS_6xx	46	1	BSEVA_L1-S	6	[31, 32, 33, 34, 35, 36]	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	51.0
Water	263.5
NEB BsaI-HFv2	17.0
Promega T4 DNA Ligase	8.5

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:

Protocol for BasicBuild 26-08-2021

Temperature (°C)	Time	
37	2 min	
20	1 min	x20 cycles
37	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	BASIC_SEVA_15.10	[1, 2, 3]	nan
2	BASIC_SEVA_16.10	[1, 4, 3]	nan
3	BASIC_SEVA_17.10	[1, 5, 6]	nan
4	BASIC_SEVA_17_TS.1	[1, 7, 6]	nan
5	BASIC_SEVA_18.10	[1, 8, 9]	nan
6	BASIC_SEVA_19.10	[1, 10, 3]	nan
7	BASIC_SEVA_25.10	[11, 2, 3]	nan
8	BASIC_SEVA_26.10	[11, 4, 3]	nan
9	BASIC_SEVA_27.10	[11, 5, 6]	nan
10	BASIC_SEVA_27_TS.1	[11, 7, 6]	nan
11	BASIC_SEVA_28.10	[11, 8, 9]	nan

Protocol for BasicBuild 26-08-2021

12	BASIC_SEVA_29.10	[11, 10, 3]	nan
13	BASIC_SEVA_35.10	[12, 2, 3]	nan
14	BASIC_SEVA_36.10	[12, 4, 3]	nan
15	BASIC_SEVA_37.10	[12, 5, 6]	nan
16	BASIC_SEVA_37_TS.1	[12, 7, 6]	nan
17	BASIC_SEVA_38.10	[12, 8, 9]	nan
18	BASIC_SEVA_39.10	[12, 10, 3]	nan
19	BASIC_SEVA_45.10	[13, 2, 3]	nan
20	BASIC_SEVA_46.10	[13, 4, 3]	nan
21	BASIC_SEVA_47.10	[13, 5, 6]	nan
22	BASIC_SEVA_47_TS.1	[13, 7, 6]	nan
23	BASIC_SEVA_48.10	[13, 8, 9]	nan
24	BASIC_SEVA_49.10	[13, 10, 3]	nan
25	BASIC_SEVA_55.10	[14, 2, 3]	nan
26	BASIC_SEVA_56.10	[14, 4, 3]	nan
27	BASIC_SEVA_57.10	[14, 5, 6]	nan
28	BASIC_SEVA_57_TS.1	[14, 7, 6]	nan
29	BASIC_SEVA_58.10	[14, 8, 9]	nan
30	BASIC_SEVA_59.10	[14, 10, 3]	nan
31	BASIC_SEVA_65.10	[15, 2, 3]	nan
32	BASIC_SEVA_66.10	[15, 4, 3]	nan
33	BASIC_SEVA_67.10	[15, 5, 6]	nan
34	BASIC_SEVA_67_TS.1	[15, 7, 6]	nan
35	BASIC_SEVA_68.10	[15, 8, 9]	nan
36	BASIC_SEVA_69.10	[15, 10, 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. Below gives an example for a 3-part assembly:

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

Protocol for BasicBuild 26-08-2021

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.