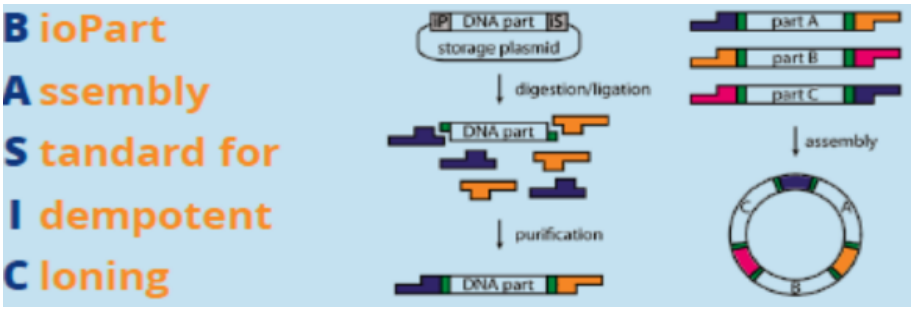
Protocol for BasicBuild 03-11-2021



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 mass for 30 µL clip reaction (ng)	Suffix ID	Total assemblies	Assembly indexes	Clip plate mapping
1	LMP-P	BS_1xx	57	BSEVA_L1-S	5	[1, 2, 3, 4, 5]	nan
2	BSEVA_L1-P	BS_x5ax	237	LMS-S	6	[1, 6, 11, 16, 21, 26]	nan
3	LMS-P	B407_J23119-RBS34-mScarl	148	LMP-S	12	[1, 2, 6, 7, 11, 12, 16, 17, 21, 22, 26, 27]	nan
4	BSEVA_L1-P	BS_x6x	100	LMS-S	6	[2, 7, 12, 17, 22, 27]	nan
5	BSEVA_L1-P	BS_x7x	134	LMS-S	6	[3, 8, 13, 18, 23, 28]	nan
6	LMS-P	B408_J23119-RBS-A12-mSc	157	LMP-S	12	[3, 5, 8, 10, 13, 15, 18, 20, 23, 25, 28, 30]	nan
7	BSEVA_L1-P	BS_x9x	131	LMS-S	6	[4, 9, 14, 19, 24, 29]	nan

8	LMS-P	B405_J23106-RBS34-mScarl	148	LMP-S	6	[4, 9, 14, 19, 24, 29]	nan
9	BSEVA_L1-P	BASIC_construct_BS_x7x_	137	LMS-S	6	[5, 10, 15, 20, 25, 30]	nan
10	LMP-P	BS_2xx	51	BSEVA_L1-S	5	[6, 7, 8, 9, 10]	nan
11	LMP-P	BS_3xx	45	BSEVA_L1-S	5	[11, 12, 13, 14, 15]	nan
12	LMP-P	BS_4xx	55	BSEVA_L1-S	5	[16, 17, 18, 19, 20]	nan
13	LMP-P	BS_5axx	67	BSEVA_L1-S	5	[21, 22, 23, 24, 25]	nan
14	LMP-P	BS_6xx	46	BSEVA_L1-S	5	[26, 27, 28, 29, 30]	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	45.0
Water	232.5
NEB BsaI-HFv2	15.0
Promega T4 DNA Ligase	7.5

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	BASIC_SEVA_15a.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_19.10	[1, 7, 8]	nan
5	BASIC_SEVA_17_pKD46.10	[1, 9, 6]	nan
6	BASIC_SEVA_25a.10	[10, 2, 3]	nan
7	BASIC_SEVA_26.10	[10, 4, 3]	nan
8	BASIC_SEVA_27.10	[10, 5, 6]	nan
9	BASIC_SEVA_29.10	[10, 7, 8]	nan
10	BASIC_SEVA_27_pKD46.10	[10, 9, 6]	nan
11	BASIC_SEVA_35a.10	[11, 2, 3]	nan
12	BASIC_SEVA_36.10	[11, 4, 3]	nan
13	BASIC_SEVA_37.10	[11, 5, 6]	nan
14	BASIC_SEVA_39.10	[11, 7, 8]	nan
15	BASIC_SEVA_37_pKD46.10	[11, 9, 6]	nan
16	BASIC_SEVA_45a.10	[12, 2, 3]	nan
17	BASIC_SEVA_46.10	[12, 4, 3]	nan
18	BASIC_SEVA_47.10	[12, 5, 6]	nan
19	BASIC_SEVA_49.10	[12, 7, 8]	nan
20	BASIC_SEVA_47_pKD46.10	[12, 9, 6]	nan
21	BASIC_SEVA_5a5a.10	[13, 2, 3]	nan
22	BASIC_SEVA_5a6.10	[13, 4, 3]	nan
23	BASIC_SEVA_5a7.10	[13, 5, 6]	nan
24	BASIC_SEVA_5a9.10	[13, 7, 8]	nan
25	BASIC_SEVA_5a7_pKD46.10	[13, 9, 6]	nan

26	BASIC_SEVA_65a.10	[14, 2, 3]	nan
27	BASIC_SEVA_66.10	[14, 4, 3]	nan
28	BASIC_SEVA_67.10	[14, 5, 6]	nan
29	BASIC_SEVA_69.10	[14, 7, 8]	nan
30	BASIC_SEVA_67_pKD46.10	[14, 9, 6]	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.