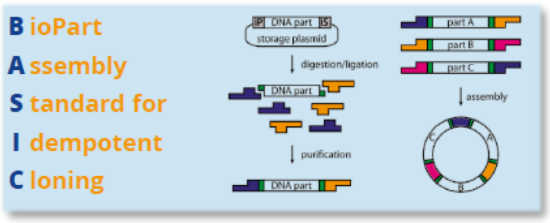
BASIC protocol version 17/08/2018



This protocol describes the BASIC assembly method assuming the use of linkers provided by Biolegio and BASIC parts stored in backbones free of BsaI sites.

0. Preparation of BASIC linkers and BASIC bioparts

BASIC linkers can be ordered from Biolegio (*info@biolegio.com*) and will be delivered in a lyophilized format along with linker annealing buffer. Once restored to 200 µl, a given BASIC linker solution is sufficient for 200 BASIC linker reactions, each of which can be used for the construction of up to 30 plasmids.

New linkers are ready to use in a few simple steps and may be stored at -20°C for up to 3 months:

1. Spin down tubes with lyophilized linkers to ensure oligos are at bottom of the tube.
2. Set heating block to 95°C.
3. Add 200 µl of linker annealing buffer to each linker tube and leave on bench for 1h.
4. Vortex tubes and collect liquid at tube bottom via a quick centrifuge spin.
5. After heating block reached 95°C, place tubes into block and slightly loosen tube caps to allow for heat expansion.
6. After 5 min switch of heating block and tighten tube caps again to avoid evaporation.
7. Allow tubes to cool down to room temperature over at least 1h in heat block.
8. Collect solution at tube bottom via a quick centrifuge spin.
9. Linkers are ready to use or can be stored at -20°C until (next) use.

Typically, bioparts for BASIC assembly are provided in storage plasmids. For each BASIC linker ligation reaction 50 ng of plasmid per 1kb of total plasmid size (including BASIC part and storage backbone) are required. Usually, that amount of DNA is provided in 1 µl of a typical miniprep of biopart storage plasmids (200 ng/µl for a 4kb plasmid).
If PCR products or gene fragments are used as reaction input, 50 ng per 1kb linear DNA are required.

I. BASIC reaction (5 min hands on time + 90 min reaction)

For each BASIC linker ligation reaction, setup 1 PCR tube with 30 µl total volume:

Reagent	Volume
dH_20	17 µl
Promega T4 buffer (10x)	3 µl
Prefix Linker	1 µl
Suffix Linker	1 µl
BASIC biopart	0.5-6 µl (50 ng per 1kb total plasmid size)
dH_20	add dH_20 to reach 28.5 µl volume
NEB BsaI-HF v2 enzyme (R3733) 20 U/µl	1 µl
Promega T4 ligase (M1801) 1-3 U/µl	0.5 µl
	Mix by pipetting up and down

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

Temperature	Time	
37°C	2 min	**X 20 cycles**
20°C	1 min	
55°C	5 min	
(80°C)	(20 min)	optional

II. Magbead purification (20 min hands on time)

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.
We recommend using a 96 well Falcon plate (Falcon 351177) in combination with an Ambion magnetic plate (AM10050) for quick magbead immobilisation and easy pipetting access.

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 5min to allow DNA binding to magbeads.
3. Place Falcon plate on magnetic stand and wait for rings to form and solution to clear.
4. Remove the solution with a 200 µl pipette tip from the centre of each well.
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 dH_20.
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. Pipette 30 µl of H_20 with eluted DNA into fresh 1.5 ml Eppendorf tube for direct use in assembly or storage at -20°C for up to 1 month.

III. Assembly reaction (5 min hands on time + 45 min reaction)

For each BASIC assembly (example here with 3 parts) combine parts with buffer in a PCR tube:

Reagent	Volume
dH_20	2 µl
NEB CutSmart buffer 10x	1 µl
Linker ligated BASIC part A	1 µl
Linker ligated BASIC part B	1 µl
Linker ligated BASIC part C	1 µl
dH_20	Top up to 10 µl total volume

Run assembly reaction in PCR machine with following programme:

Temperature	Time
50°C	45 min
4°C	hold

IV. Transformation (5 min hands on time + 90 min incubation time)

Use 50 µl of chemically competent cells DH5alpha with high transformation efficiency (10^9 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 Eppendorf 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.

Reagents and equipment required

Item	Order number
PCR machine	
Heat block (up to 95°C) fitting 2ml Eppendorf tubes	
Water bath (42°C) for transformation	
Magnetic plate	Ambion AM10050 (Thermo)
96 well U-Bottom Falcon plate	Falcon 351177 (Thermo)
Eppendorf tubes	
PCR tubes	
Pipettes/tips 10 and 200 µl	
Magnetic beads	Ampliclean (Nimagen)
dH_20	
70% EtOH	Freshly made
Biolegio BASIC linkers	BBT-18100 (Biolegio)
BASIC parts in storage plasmids (200ng/µl)	
NEB BsaI-HF v2 enzyme (R3733) 20 U/µl; includes CutSmart buffer	R3733 (NEB)
Promega T4 ligase (M1801) 1-3U/µl; includes Promega T4 buffer	M1801 (Promega)
Chemically competent cells (DH5alpha, $1x10^9$ CFU/µg pUC19); includes SOC media	C2987I (NEB)