GenBuilder™ Cloning Kit Protocol

Product information

The GenBuilder Cloning Kit enables seamless and one-step cloning of multiple DNA fragments into a linearized vector. The proprietary enzyme mix fuses together DNA fragments efficiently and precisely by recognizing 15-40 bp overlaps at the end of each fragment. The system allows the cloning of the DNA fragments into any linearized vector, requires no pre-existing recombination sites or helper sequences, and eliminates the need for complicated restriction and ligation process. The GenBuilder Cloning kit is recommended for simultaneous cloning of up to five DNA fragments. For a greater number of fragments, or for assembling ssDNA oligos with dsDNA fragments, the GenBuilder Plus Cloning kit (L00744) is recommended.

Kit contents and Storage

The kit contains GenBuilder 2X Master Mix and Positive Control

Keep all components at –20°C for long term storage. Thaw the master mix on ice prior to use. For the best performance, please aliquot the master mix into smaller volumes and avoid unnecessary freeze-thaw cycles. The GenBuilder 2X Master Mix is stable at 4°C for at least two weeks.

Calculate input DNA quantity

Option 1. We recommend adding 0.1 pmol of each DNA fragment in an assembly reaction.

- Use 2:1 insert to vector molar ratio for cloning one or two fragments.
- Use 1:1 insert to vector molar ratio for cloning more than two fragments.
- Use 5 times more inserts when the insert is less than 200 bp.

Determine the concentration of your DNA fragment solutions by UV or by fluorescence. For 0.1 pmol of each DNA fragment, use the concentration (ng/µl) and length (bp) of the DNA fragment to calculate the volume required for the GenBuilder reaction:

$$\mu l\ of\ DNA\ fragment = \frac{0.65 \times pmol \times bp}{ng/\mu l}$$

Option 2. You can use the following table for a quick estimation of input DNA concentration in GenBuilder reactions (example: 0.1 pmol fragment in the reaction):

Fragment length	Input DNA
0.5 kb	33 ng
1 kb	67 ng
1.5 kb	100 ng
2 kb	133 ng

3 kb	200 ng
5 kb	330 ng

GenBuilder assembly reaction protocol

Note: Please download the comprehensive protocol at: http://www.genscript.com/genbuilder-dna-assembly.html

1. Set up the following reaction on ice:

	Assembly reaction	Positive control
Each DNA fragment for assembly	0.1 pmol * X µl	10 µl
Linearized vector	0.1 pmol X µl	N/A
GenBuilder 2x Master Mix	10 µl	10 µl
Deionized H_2O	to 20 µl	-

* If unpurified PCR product is used for assembly, the total volume of PCR product should not exceed 10% of the total reaction volume.

2. Gently mix the reactions by pipetting.
3. Incubate the reaction in a thermocycler at 50°C for 15 minutes. For DNA assembly reaction involving more than six fragments, the incubation time may be increased to 60 minutes.
4. Transform 2 µl of the assembly product into competent *E. coli* cells. For electroporation, dilute the reaction product 5-fold and use 1 µl for transformation. It is recommended to use competent cells with high efficiency (>2×10^8 transformants per µg pUC19 plasmid).
5. Spread 1/10 volume of the recovered cells onto selection plates. For a complex assembly (cloning more than 3 fragments), we recommend that you concentrate the cells before spreading on selection plates. For a positive control reaction, spread 1/10 volume of the cells on LB Agar plates containing 100 mg/ml ampicillin and 0.1 mM IPTG.
6. Incubate the plate overnight at 37°C. A successful positive control reaction should reconstitute red fluorescent protein (RFP) and produce hundreds of red colonies on selective plates.

GenScript USA Inc,
Email: product@genscript.com
Web: http://www.genscript.com
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