

Gibson Assembly (NEBuilder)

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Abstract

Gibson assembly using NEBuilder HiFi DNA assembly kit. Specification of the original NEB protocol.

Citation: Snehadri Sinha Gibson Assembly (NEBuilder). **protocols.io**

dx.doi.org/10.17504/protocols.io.gzebx3e

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Protocol

Step 1.

Set up assembly reaction on ice:

	2-3 Fragment Assembly
Recommended DNA Ratio	vector:insert = 1:2
DNA	X µl (50 ng vector, insert(s) based on that)
NEBuilderHiFi DNA Assembly Master Mix	10 µl
H2O	10-X µl
Total Volume	20 µl

Step 2.

Incubate samples in a thermocycler at 50 C for 60 minutes

Step 3.

Store samples on ice or -20 C for subsequent transformation.

Step 4.

Transform 2 µL of assembled product into E. coli competent cells. For 10-beta E. coli cells provided with the kit, use NEBuilder transformation protocol.