

content tester

Alexei Stoliartchouk

Abstract

The Gibson Assembly[®] HiFi 1 Step method utilizes a blend of enzymes to assemble the DNA quickly and efficiently. The use of a High-Fidelity DNA polymerase enzyme allows for error free cloning resulting in a seamless construct ready for multiple downstream applications. The HiFi 1 Step method allows Gibson Assembly of up to 5 fragments using a one tube, one-step, isothermal reaction. Multiple DNA fragments can be joined with greater than 90% cloning efficiency. Large constructs up to 100kb in length can be efficiently assembled in multi stage reactions.

Citation: Alexei Stoliartchouk content tester. **protocols.io**

dx.doi.org/10.17504/protocols.io.d7t9nm

Published: 13 Dec 2016

Protocol

test

Step 1.

STEP ONE

<https://www.protocols.io/forvendor>

■ ANNOTATIONS

Alexei Stoliartchouk 11 Mar 2016

test

ttttt

Step 2.

Set up the following reaction

on ice (to 20µl total volume):

	Recommended Amount of Fragments Used for Assembly		
	2-3 Fragment Assembly*	4-6 Fragment Assembly**	Positive Control†
Recommended DNA Ratio	vector:insert = 1:2	vector:insert = 1:1	
Total Amount of Fragments	0.03-0.2 pmols*	0.2-0.5 pmols**	10 µl
	X µl	X µl	
NEBuilder	10 µl	10 µl	10 µl
HiFi DNA Assembly Master Mix			
Deionized H2O	10-X µl	10-X µl	0
Total Volume	20 µl † †	20 µl † †	20 µl

testing tall MCE container

MCE

PROTOCOL

. [E2621 DNA Assembly Reaction](#)

CONTACT: [New England Biolabs](#)

Step 2.1.

Vector DNA

ANNOTATIONS

Shine Sun 19 Apr 2016

pLenti Guide puro

Weijun Liu 15 Aug 2016

pLentiCRISPRV2

Step 2.2.

Insert fragments DNA

Step 2.3.

NEBuilder HiFi DNA Assembly Master Mix



REAGENTS

 NEBuilder HiFi DNA Assembly Master Mix - 10 rxns [E2621S](#) by [New England Biolabs](#)

Step 2.4.

Deionized H2O

Step 3.

<https://www.protocols.io/forvendors>

ANNOTATIONS

Alexei Stoliartchouk 11 Mar 2016

test

Step 4.

Incubate samples in a thermocycler at 50°C for 15 minutes (when 2 or 3 fragments are being assembled) or 60 minutes (when 4-6 fragments are being assembled).