NEBuilder® Protocol Calculator

v1.1.3

ABOUT HELP

Use the NEBuilder® Protocol Calculator to calculate the optimal amounts of input DNA sequences given the length and concentration of each input fragment.

ENTER FRAGMENTS FOR ASSEMBLY

Vector	Name	Length	Concentration	
☐	ID/Name	basepairs	ng/µl	Add Fragment
☑	backbone	2682	100	Delete
☐	insert	905	10	Delete

Clear All Fragments

SUGGESTED PROTOCOL

1. Set up the reaction on ice (see table).

Component	Volume	Amount
backbone	0.8 µl	0.050 pmoles
insert	5.6 µl	0.100 pmoles
Deionized H_2O	3.6 µl	---
NEBuilder HiFi DNA Assembly Master Mix	10.0 µl	---
Total	20.0 µl	0.150 pmoles

☐ Maximize

2. Incubate samples in a thermocycler at 50°C for 15 minutes. Following incubation, store samples on ice or at –20°C for subsequent transformation.
   Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases.

3. Transform NEB® 5-alpha, NEB Stable or NEB 10-beta Competent *E. coli* cells (provided in the cloning kit, bundle or purchased separately from NEB) with 2.0 µl (10%) of the chilled assembled product, following the transformation protocol.

Related Resources

NEBuilder® HiFi DNA Assembly Reaction Protocol
NEBuilder® HiFi DNA Assembly

Related Products

NEBuilder® HiFi DNA Assembly Master Mix
NEBuilder® HiFi DNA Assembly Cloning Kit
NEBuilder® HiFi DNA Assembly Bundle for Large Fragments

Related Tools

NEBuilder® Assembly Tool