BioBrick™ Assembly Kit

1-800-632-7799
info@neb.com
www.neb.com

E0546S 001120914091

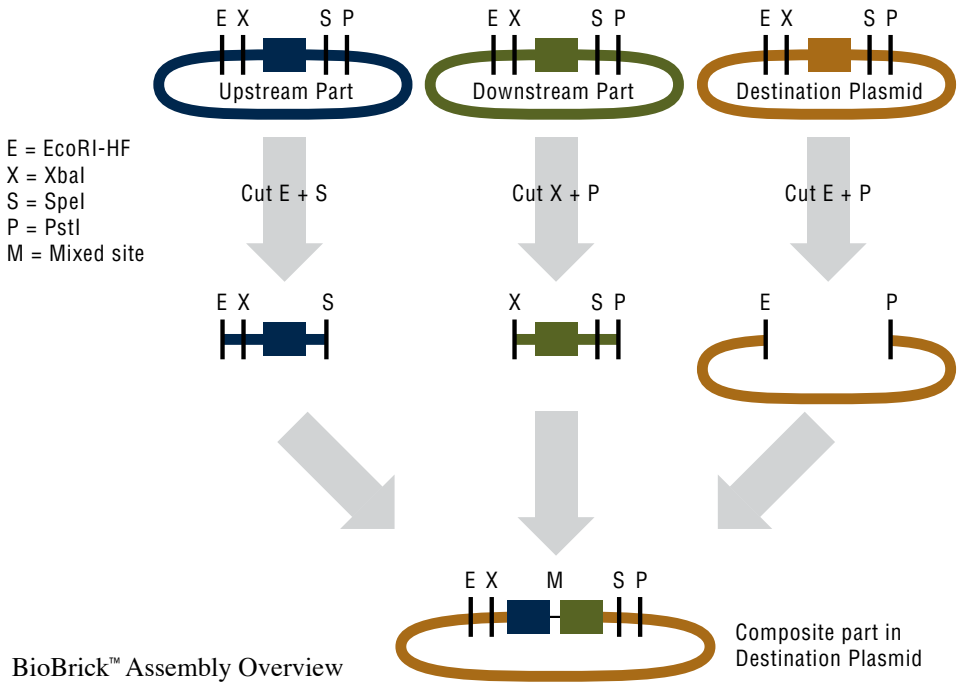
E0546S

50 reactions **Lot: 0011209**

RECOMBINANT **Exp: 9/14**

Description: The BioBrick™ Assembly Kit provides a streamlined method for assembly of BioBrick™ parts into multi-component genetic systems. BioBrick™ parts are DNA sequences that encode a defined biological function and can be readily assembled with any other BioBrick™ part. The process for assembling any two BioBrick™ parts is identical and results in a new composite BioBrick™ part.

The BioBrick™ Assembly Kit contains EcoRI-HF™, XbaI, SpeI, PstI, T4 DNA Ligase, NEBuffer 2 and BSA.



BioBrick™ Assembly Overview

The BioBrick™ Assembly Kit was developed in partnership with Ginkgo BioWorks. What follows is an abbreviated set of protocols for the use of the BioBrick™ Assembly Kit. **For more details and for technical questions, please see http://ginkgobioworks.com/support.**

To assemble an Upstream Part with a Downstream Part into a Destination Plasmid:

Digestion Protocol

Digest Upstream Part with EcoRI-HF™ and SpeI.

Upstream Part Plasmid:	500 ng
EcoRI-HF:	1 µl
SpeI:	1 µl
10X NEBuffer 2:	5 µl
100X BSA:	0.5 µl
H_2O:	to 50 µl

Digest Downstream Part with XbaI and PstI.

Downstream Part Plasmid:	500 ng
XbaI:	1 µl
PstI:	1 µl
10X NEBuffer 2:	5 µl
100X BSA:	0.5 µl
H_2O:	to 50 µl

CERTIFICATE OF ANALYSIS

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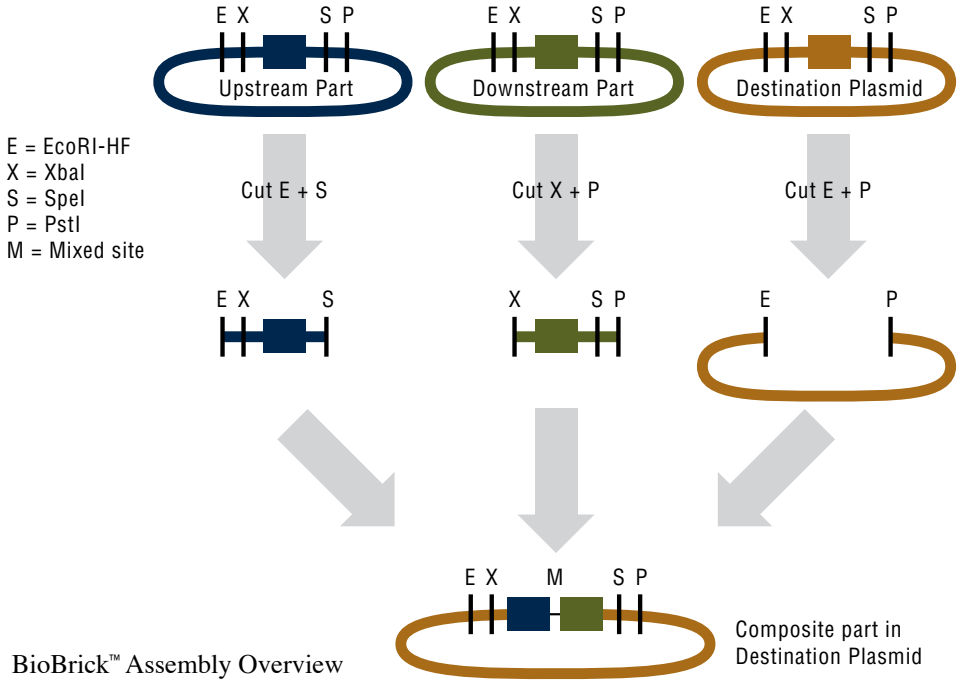

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PstI:	1 µl
10X NEBuffer 2:	5 µl
100X BSA:	0.5 µl
H_2O:	to 50 µl

CERTIFICATE OF ANALYSIS

Digest the Destination Plasmid with EcoRI-HF and PstI: The Destination Plasmid DNA should either be prepared with PCR or contain a toxic gene (e.g. ccdB, sacB) in the cloning site to avoid the need for gel purification. The Destination Plasmid should also have a different antibiotic resistance marker from both the plasmid containing the Upstream Part and the plasmid containing the Downstream Part to avoid the need to purify the Upstream and Downstream Parts.

Destination Plasmid DNA:	500 ng
EcoRI-HF:	1 µl
PstI:	1 µl
10X NEBuffer 2:	5 µl
100X BSA:	0.5 µl
H_2O:	to 50 µl

Incubate all three restriction digest reactions at 37°C for 10 minutes and then heat inactivate at 80°C for 20 minutes.

Ligation Protocol

Ligate the Upstream and Downstream Parts into the digested Destination Plasmid.

Upstream Part digestion:	2 µl
Downstream Part digestion:	2 µl
Destination Plasmid digestion:	2 µl
10X T4 DNA Ligase Buffer:	2 µl
T4 DNA Ligase:	1 µl
H_2O:	11 µl

Incubate at room temperature for 10 minutes and then heat inactivate at 80°C for 20 minutes.

Transform 2 µl of the ligation product into 50 µl of competent *E. coli* cells (or other suitable host strain). Select using the antibiotic corresponding to the Destination Plasmid.

Please refer to the individual datacards for each reagent for recommended use and storage conditions.

Technical questions regarding the individual use of any of the enzymes included in this kit should be directed to New England Biolabs by contacting 1-800-632-7799 or info@neb.com.

Technical questions regarding the assembly of BioBrick™ parts should be directed to Ginkgo BioWorks at http://ginkgobioworks.com/support.

Digest the Destination Plasmid with EcoRI-HF and PstI: The Destination Plasmid DNA should either be prepared with PCR or contain a toxic gene (e.g. ccdB, sacB) in the cloning site to avoid the need for gel purification. The Destination Plasmid should also have a different antibiotic resistance marker from both the plasmid containing the Upstream Part and the plasmid containing the Downstream Part to avoid the need to purify the Upstream and Downstream Parts.

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