Digestion for BioBrick Assembly Kit (E0546)

New England Biolabs

Abstract

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 (to assemble an Upstream Part with a Downstream Part into a Destination Plasmid). For more details and for technical questions, please see here.

Citation: New England Biolabs Digestion for BioBrick Assembly Kit (E0546). **protocols.io** dx.doi.org/10.17504/protocols.io.cphvj5
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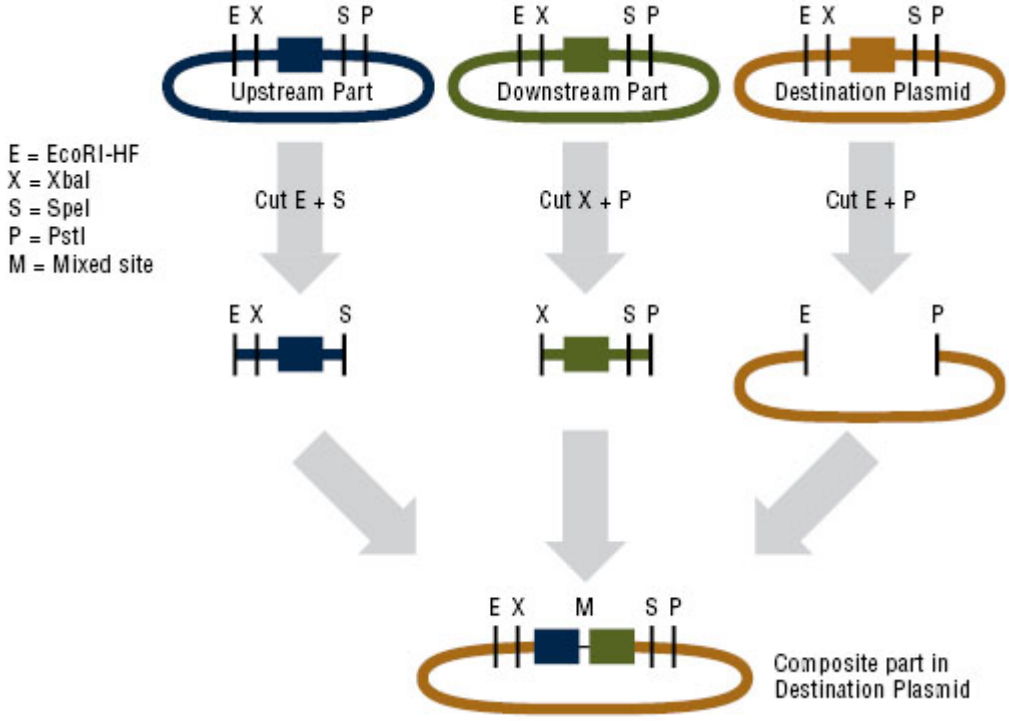
Guidelines

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 and NEBuffer 2.1.



BioBrick Assembly Overview

Materials

BioBrick Assembly Kit - 50 rxns E0546S by New England Biolabs

Protocol

Step 1.
Digest Upstream Part with EcoRI-HF™ and SpeI.

PROTOCOL

. BioBrick E0546 Upstream Reaction

CONTACT: New England Biolabs
Step 1.1.
Upstream Part Plasmid, 500 ng

AMOUNT
500 ng Additional info:
Step 1.2.
EcoRI-HF™, 1 µl

AMOUNT
1 µl Additional info:
Step 1.3.
SpeI, 1 µl

AMOUNT
1 µl Additional info:
Step 1.4.
10X NEBuffer 2.1, 5 µl

AMOUNT
5 µl Additional info:
Step 1.5.
H2O to 50 µl

Step 2.
Digest Downstream Part with XbaI and PstI.

PROTOCOL

. BioBrick E0546 Downstream Reaction

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NOTES
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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.

Step 2.1.
Upstream Part Plasmid, 500 ng

AMOUNT
500 ng Additional info:

Step 2.2.
XbaI, 1 µl

AMOUNT
1 µl Additional info:

Step 2.3.
PstI, 1 µl

AMOUNT
1 µl Additional info:

Step 2.4.
10X NEBuffer 2.1, 5 µl

AMOUNT
1 µl Additional info:

Step 2.5.
H2O to 50 µl

Step 3.
Digest the Destination Plasmid with EcoRI-HF™ and PstI.

PROTOCOL

. BioBrick E0546 Destination Reaction

CONTACT: New England Biolabs

Step 3.1.
Destination Plasmid DNA, 500 ng

AMOUNT
500 ng Additional info:

Step 3.2.
EcoRI-HF™, 1 µl

AMOUNT
1 µl Additional info:

Step 3.3.
PstI, 1 µl

AMOUNT
1 µl Additional info:

Step 3.4.
10X NEBuffer 2.1, 5 µl

Step 3.5.
H2O to 50 µl

Step 4.
Incubate all three restriction digest reactions at 37°C for 10 minutes

DURATION

00:10:00

Step 5.

Heat inactivate at 80°C for 20 minutes.

DURATION

00:20:00