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Feb 02, 2022

Digestion for BioBrick Assembly Kit (E0546) V.2

New England Biolabs[1]

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This protocol explains methods for assembling multi-compontent genetic systems using BioBrick® parts.

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Biobrick, Assembly, E0546

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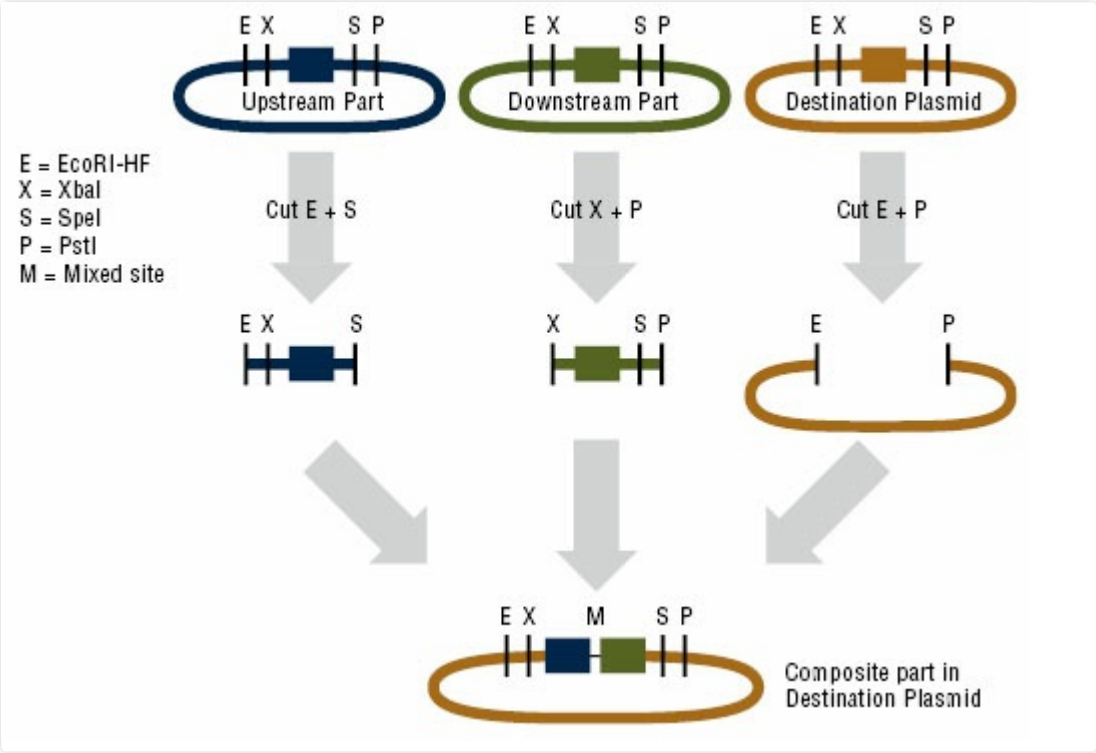
The BioBrick® Assembly Kit provides a streamlined method for assembly of BioBrick parts into multi-component genetic systems. BioBrick parts are DNA sequences that encode a defined biological function and can be readily assembled with any other BioBrick part. The process for assembling any two BioBrick 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 **New England Biolabs Catalog #E0546S**

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

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.

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Digest Upstream Part with EcoRI-HF® and SpeI:

A	B
Reagent	**Volume**
Upstream Part Plasmid	500 ng
EcoRI-HF	1 µl
SpeI	1 µl
10X NEBuffer 2.1	5 µl
H2O	to 50 µl

2

Digest Downstream Part with XbaI and PstI:

A	B
Reagent	**Volume**
Upstream Part Plasmid	500 ng
XbaI	1 µl
PstI	1 µl
10X NEBuffer 2.1	5 µl
H2O	to 50 µl

3

Digest the Destination Plasmid with EcoRI-HF® and PstI:

A	B
Reagent	**Volume**
Destination Plasmid DNA	500 ng
EcoRI-HF	1 µl
PstI	1 µl
10X NEBuffer 2.1	5 µl
H2O	to 50 µl

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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Incubate all three restriction digest reactions at **37 °C** for **00:10:00** .

5 

Heat inactivate at **80 °C** for **00:20:00** .

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