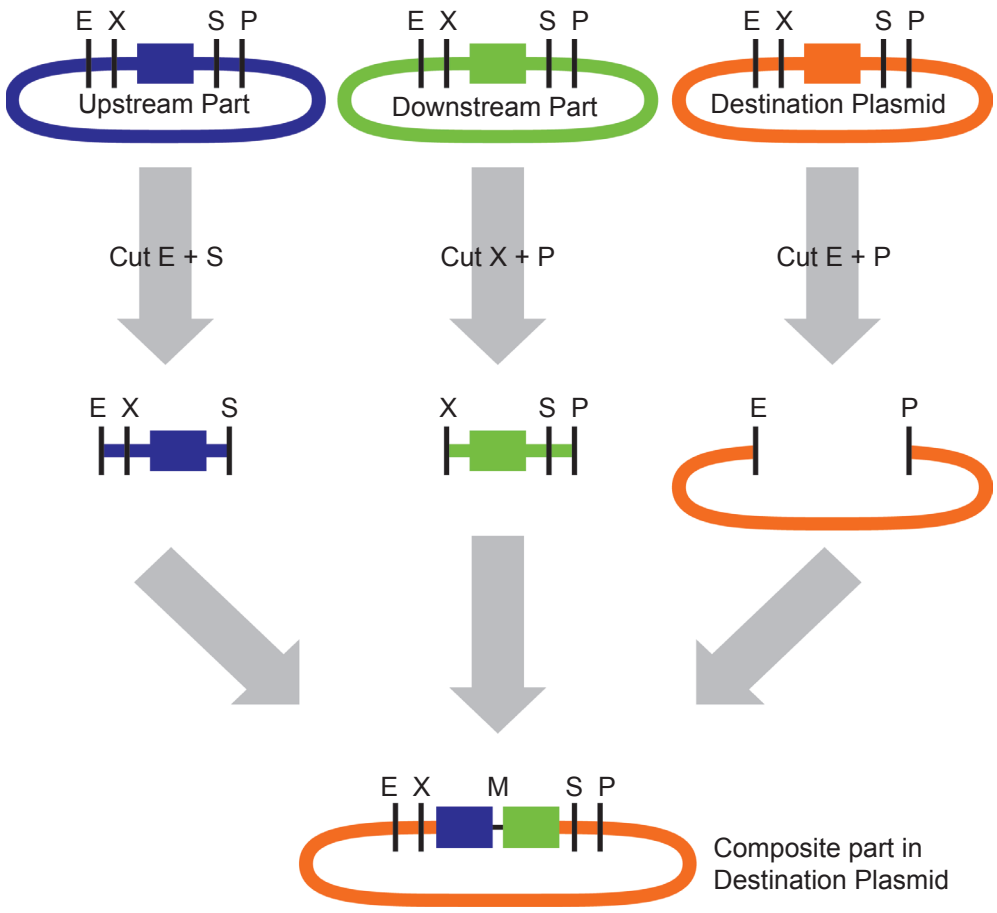
BioBrick™ Assembly Manual

ginkgobioworks™

NEW ENGLAND BioLabs Inc.

This manual describes the major steps of BioBrick assembly using BioBrick Assembly Standard 10. The input to the protocol is DNA for the two parts to be assembled and a destination plasmid. The manual includes protocols for the digestion of the three input DNA molecules and the ligation of the digested DNA to form a circularized plasmid containing the composite part. The product of the ligation reaction can be used to transform competent cells with the composite part. To read more about the BioBrick system and browse the BioBrick collection, visit the Registry of Standard Biological Parts at http://partsregistry.org.

E=EcoRI-HF™
X=XbaI
S=SpeI
P=PstI
M=Mixed site



BioBrick assembly overview

1 Start with two BioBrick parts and a BioBrick destination plasmid. The destination plasmid contains a toxic gene, *ccdB,* in the BioBrick cloning site and a different antibiotic resistance marker to the upstream and downstream parts.

2 Digest each of the parts with the appropriate restriction enzymes.

3 Mix the digests together and perform a ligation step. One of the ligation products formed will be the correctly assembled composite part in the destination plasmid. You can use the ligation mix to transform competent cells with the new composite part.

The BioBrick™ Assembly Kit from NEB and Ginkgo BioWorks has been designed for use with this manual. Download this manual from http//ginkgobioworks.com/support

Version 1.0

materials

consumables

A Restriction enzymes (EcoRI-HF, XbaI, SpeI, PstI), NEBuffer 2, BSA

B 10X T4 DNA Ligase Reaction Buffer, T4 DNA Ligase

C H_2O (not shown)

D Small PCR tubes

E 2 µl, 200µl pipet tips

F Destination plasmid as purified DNA

G Upstream and downstream parts as purified DNA

equipment

H 2 µl and 20 µl pipet

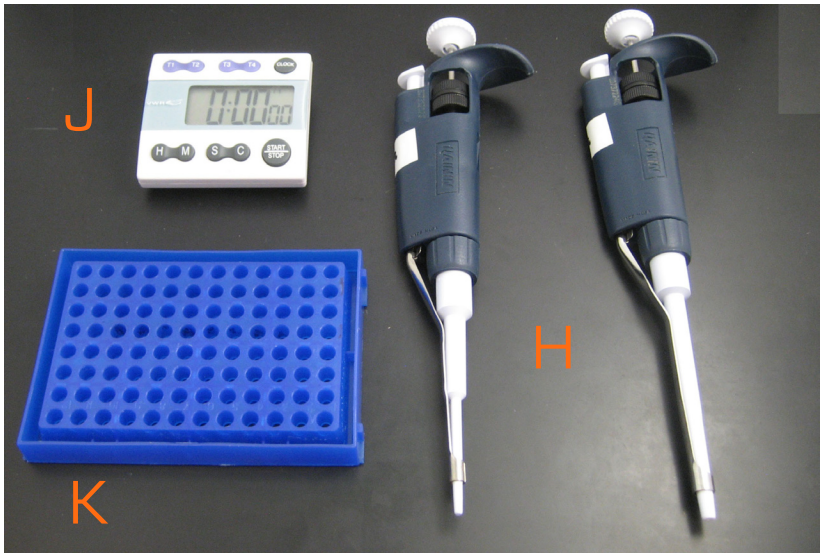
I Incubator/water bath/thermocycler capable of holding 37°C and 80°C (not shown)

J Timer

K Rack for small PCR tubes

L -20°C freezer (not shown)

M Freezer box



! Safety note: While harmless, these protocols involve recombinant DNA techniques that should only be performed in a laboratory with suitable recombinant DNA and safety permits as required by your local, state, or federal regulations.

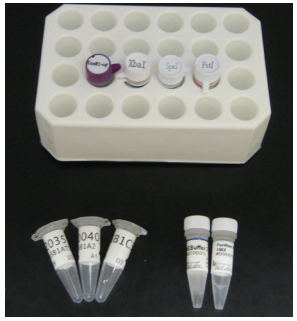
digest

! This protocol assumes you have purified DNA for each of the BioBrick parts you want to assemble and also that you have purified DNA for the destination plasmid. The DNA could be produced from a DNA miniprep or a PCR amplification from a template. If the DNA was produced via a PCR amplification, the protocol assumes the DNA has been purified from the PCR enzymes that can reduce ligation efficiency.

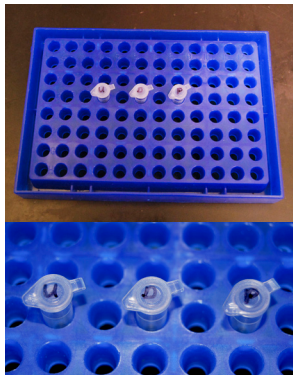
The destination plasmid must have a different antibiotic resistance than the plasmids carrying the parts to be assembled, otherwise, many of the colonies obtained after transformation of competent cells will contain the input BioBrick parts, and not the composite BioBrick part. The toxic gene in the BioBrick cloning site of the destination plasmid ensures that cells transformed with undigested destination plasmid will not grow.

prepare reaction mix

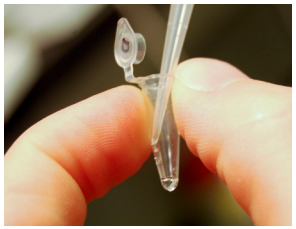
1 Remove the DNA for the upstream part, the downstream part and the destination plasmid along with NEBuffer 2 and BSA from the freezer to thaw. Thawing is fast if the tubes are immersed in room temperature water. You can also remove the enzymes from the freezer but leave them in a cold box so they remain close to -20°C.



2 You will need three PCR tubes, one for the digest of the upstream part, one for the downstream part, and one for the destination plasmid. You should label each tube (for example, U, D, P, for upstream part, downstream part, and destination plasmid respectively).



3 To each tube, add H_2O and 500 ng of the part or plasmid to be digested. Adjust the amount of water you add such that the total volume in each tube is 42.5 µl.



4 Add 5 µl of NEBuffer 2 to each tube.

5 Add 0.5 µl of BSA to each tube.

6 Add 1 µl of the first appropriate* restriction enzyme to each tube**.

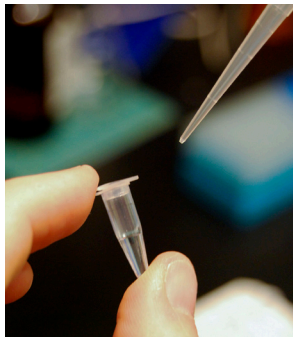
7 Add 1 µl of the second appropriate* restriction enzyme to each tube**.

* See the overview diagram on Page 1 for the appropriate restriction enzymes for each part and the destination plasmid.

** When pipeting restriction enzyme, only touch the very end of the pipet tip into the restriction enzyme. Restriction enzymes are stored in a high percentage glycerol solution that sticks to the outside of the pipet tip. If you dip the tip deeply into the restriction digest you will add much more restriction digest than needed as well as increase the glycerol concentration of the digest mix. A high glycerol concentration (>5%) can result in non-specific cutting of the DNA (referred to as "star activity").

digest

8 The total volume in each tube should now be 50 µl. Ensure the digest is well-mixed by flicking the tube. You can spin the tube in a microcentrifuge for a few seconds to collect the liquid in the bottom of the tube again.



9 Return all reagents and purified DNA to the -20°C freezer.

incubations

10 Incubate the three restriction digests at 37°C for 15 min. Either a water bath, an incubator, or a thermocycler are suitable for this incubation.

11 Incubate the three restriction digests at 80°C for 20 min to deactivate the restriction enzymes. This step is most conveniently performed in a thermocycler so choose tubes for the restriction digest that fit in your thermocycler. As an easy way to confirm the digests worked, consider running 20 µl of each digest on a 1% agarose gel and look for bands of the expected length.

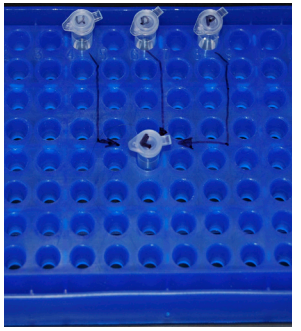
12 Store the three restriction digests at -20°C or proceed immediately to the ligation step.

ligate

prepare reaction mix

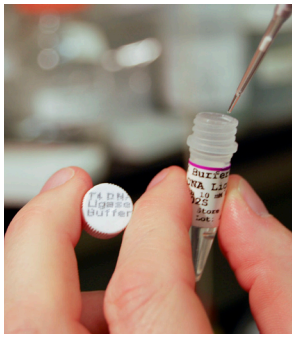
1 Remove the 10X T4 DNA Ligase Reaction Buffer* from the freezer to thaw. You can also remove the T4 DNA Ligase enzyme from the freezer at this point but leave the ligase in a cold box to keep it close to -20°C. Thawing is fast if the buffer tube is immersed in room temperature water. Once thawed, agitate the 10X T4 DNA Ligase Reaction Buffer until all precipitate goes into solution.

2 Add 11 µl of H_2O to a 200 µl PCR tube.



3 Add 2 µl from each of each of the digests to the tube**.

4 Add 2 µl of 10X T4 DNA Ligase Reaction Buffer to the tube.

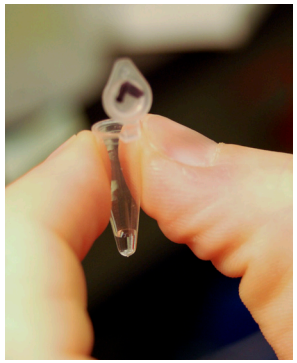


* Repeated freeze-thaw cycles of the buffer can degrade the ATP in the buffer thereby making the ligation reaction less efficient. It is wise to aliquot the buffer into 10 µl aliquots prior to freezing.
** There is no need to purify the restriction digests via gel electrophoresis or any other method.

ligate

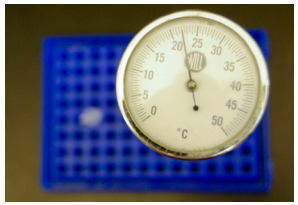
5 Add 1 µl of the T4 DNA Ligase to the tube.

6 The total volume in each tube should now be 20 µl. Ensure the ligation is well-mixed by flicking the tube. You can spin the tube in a microcentrifuge for a few seconds to collect the liquid in the bottom of the tube again.

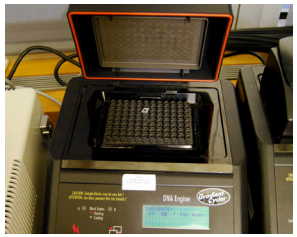


incubations

7 Incubate the reaction mix at room temperature for 10 min.



8 Incubate the reaction mix at 80°C for 20 min. The 80°C incubation deactivates the enzyme and improves transformation efficiency.



9 Store the ligation mix at -20°C or proceed immediately to the transformation step.

transformation

The ligation mix can be used to transform competent *E. coli* cells. You can purchase many kinds of competent cells. NEB 10-beta competent coli are a good choice for most applications (http://www.neb.com/nebecomm/products/productC3019.asp). For a protocol to prepare and transform competent *E. coli* cells, visit - http://openwetware.org/wiki/TOP10_chemically_competent_cells.

Remember to spread the transformed cells on plates supplemented with the antibiotic(s) to which the destination plasmid provides resistance. Any transformed destination plasmid that was not cut or was only cut with one enzyme will still contain the *ccdB* gene and cells containing those plasmids will not be able to grow.

references

1. Knight, T. F. Idempotent Vector Design for Standard Assembly of BioBricks. MIT Synthetic Biology Working Group Technical Report. http://hdl.handle.net/1721.1/21168 (2003).
2. Shetty, R. P., Endy, D., Knight, T. F. Engineering BioBrick vectors from BioBrick parts. J Biol Eng 2:5 (2008).
3. Canton, B., Labno, A., Endy, D. Refinement and standardization of synthetic biological parts and devices. Nat Biotech 26, 787-793 (2008).
4. This manual and further technical support is available from http://ginkgobioworks.com/support.