Ligase-Independent Cloning as a Standard for BioBrick Preparation

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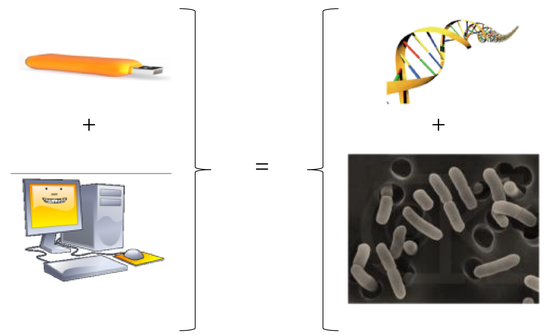
Rationale

The underlying idea of iGEM is that an engineering approach can be applied to biology. The ultimate goal is to have software (DNA sequences) that can be run on compatible hardware (microorganisms) anywhere in the world.

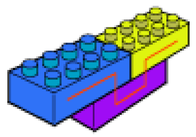


The portability of such a biological hardware-software interface requires extensive standardization. The requisite standards exist for :

- the definition of BioBricks; and,
- the use of BioBrick parts in composite devices.

There is not yet a standard method, though, for constructing new BioBricks.

Ideally, a standard method for constructing BioBricks should be highly portable, but also *simple*. The BioBricks used by iGEM are advertised with Lego® blocks, implying a very easy-to-use set of tools. Our team of high school students discovered that synthetic biology is not (yet, at least) at the stage of quick and easy implementation in just anyone's kitchen or garage.



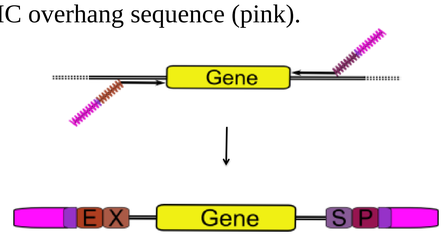
Therefore, we chose to investigate ligase-independent cloning (LIC). Our approach was based on a method described by Aslanidis & de Jong (1990). We believe that this technique can serve as a standard for BioBrick construction. It is relatively easy to implement, and also lends itself to automation in the future, which contributes to the engineering approach promoted by iGEM.

The LIC Method

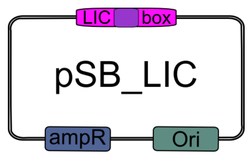
Goal: create a standard BioBrick from any DNA sequence of interest.

1.) Select Gene
Identify a gene (DNA sequence) of interest

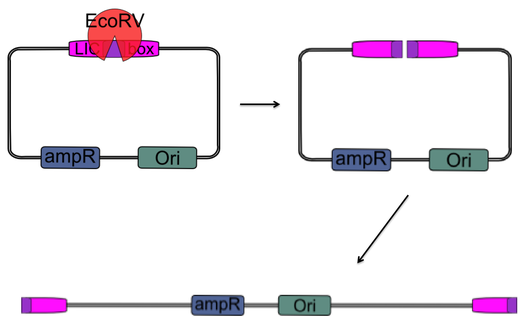
2.) Amplify Gene
Amplify the DNA sequence of interest, using PCR primers containing the BioBrick prefix (brown; EcoRI & XbaI) & suffix (purple; SpeI & PstI) as well as a LIC overhang sequence (pink).



3.) Obtain LIC plasmid
Obtain pSB_LIC, Registry part K155000.



4.) Digest with EcoRV
Digest pSB_LIC with EcoRV.



5.) Digest with T4 polymerase
Digest linearized pSB_LIC, along with desired insert sequence, with T4 DNA polymerase. This digestion is done in the presence of only one dNTP, complementary to a base absent from the LIC overhang. (for the overhang below right, only dCTP is supplied).

```
5'-ATCCCTACTACCTACG---3'
3'-TAGGGATGATGGATGC---5'
          ↓
5'-ATCCCTACTACCTACG---3'
                3'-C---5'
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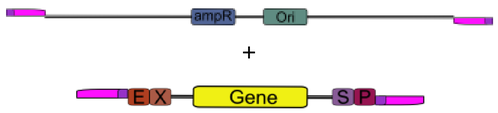
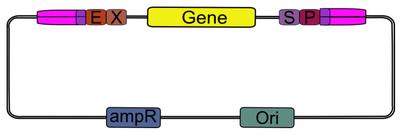
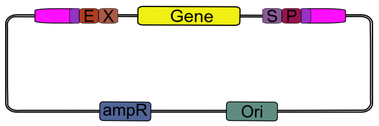
Therefore, polymerase activity is impossible, and the 3' - 5' exo-nuclease activity of T4 DNA polymerase predominates. The 3' end of each DNA strand is chewed away, until an appropriate base is reached at the end of the LIC overhang (C, above). The dNTP required to base-pair with this base is present in the digestion buffer, and so the polymerase activity now balances the exonuclease activity, ending net digestion.



6.) Mix Plasmid & Insert
Mix T4 polymerase-treated pSB_LIC and insert, allowing complementary LIC overhangs to base-pair. The long base-paired regions are sufficient to hold them together through transformation, after which *E. coli* 's own ligase will repair the DNA backbone nicks.

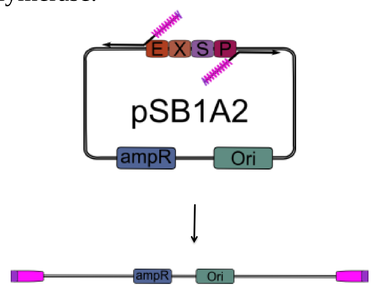


7.) Transform & Use BioBrick
The DNA sequence of interest, with standard BioBrick prefix and suffix, is now ready for further use.



Preparative Work

We obtained pSB_LIC by round-the-world PCR amplification from pSB1A2, using a high-fidelity DNA polymerase.



The linear PCR product was then blunt-end ligated to yield the circular plasmid submitted as part K155000.

We attempted the preparation of GFP and of xylE (for the U of Lethbridge team) using the LIC method, but were unable to confirm correct transformation due to time limitations. However, agarose gel and DNA sequencing analyses suggest that pSB_LIC has been satisfactorily prepared, and is ready for use.

Please feel free to use it for next year's competition!

Reference

Aslanidis, C. & de Jong, P.J. (1990) "Ligation-independent cloning of PCR products (LIC-PCR)." *Nucl. Acids Res.* **18**:20, 6069-6074.

Acknowledgements

Thanks to the University of Lethbridge Faculty of Arts & Science, to the Department of Chemistry & Biochemistry, to Dr. Brent Selinger (Biological Sciences), and to the University of Lethbridge iGEM team.