BioBrick Standard

Introduction

Composability

The BioBrick standard is a format of DNA construction which allows for "composability". An illustration of composability …

- DNA sequences 'A' and 'B' can be ligated together using the procedure 'X', into a composite product 'AB'.
- DNA sequences 'C' and 'D' can also be ligated together using the same procedure 'X', into some other composite product 'CD'.
    - NOTE that this is uncommon – usually, manipulation of restriction sites has to be customized according to the specifics of each ligation.
- FINALLY, composite sequences 'AB' and 'CD' can be ligated together, once again using the same procedure 'X'.

This ability to use procedure X to form composites under any situation is called "composability".

Basics of the Standard

DNA pieces which comply with the BioBrick standard have several characteristics …

- The coding sequence, or "the part", never has restriction sites for any of the following for enzymes: EcoR1, Xba1, Spe1, and Pst1.
- Immediately outside the part, however, these four restriction sites are found in the above order: E and X before the part (called the **'prefix'**), and S and P after the part (called the **'suffix'**).

Procedure 'X': 3-way Ligation

- **Example:** you are trying to construct the composite part AB
- Digest the plasmid containing part A with EcoR1 and Spe1.
- Digest the plasmid containing part B with Xba1 and Pst1.
- Digest a new plasmid backbone (which also contains the four restriction sites, but without any parts in between) with EcoR1 and Pst1.
- Ligation results in …
    - EcoR1 overhang of the plasmid backbone anneals to the EcoR1 overhang of the part A.
    - Spe1 overhang of part A anneals to the Xba1 overhang of the part B, forming a mixed site which can now no longer be cut by either enzyme (this is the special feature which the BioBricks standard exploits) – the site between the two parts, which contains the mixed site, is called 'the scar'.
    - Pst1 overhang of part B anneals to the Pst1 overhang of the plasmid backbone.
- **Final product:** E, X, part 1, scar, part 2, S, P – NOTE that this is once again in the BioBrick format.

Finer Features of the Ligation Procedure

- **The Death Gene:** the plasmid backbone contains a death gene (ccdB gene) between the E and P sites which, when expressed, is toxic to all strains of *E. coli* except for DB3.1. As such, when you transform the ligation products into non-resistant strains of *E. coli*, only recombinant colonies will grow, since the ccdB gene shall have been disrupted in those plasmids.
- **RFP Gene:** alternatively, some backbones are also given which have the RFP gene in place of the ccdB gene.
- **Antibiotic resistance:** three different antibiotic resistances are used in BioBrick plasmids, allowing you to select for the resistance detail within the backbone region of the new plasmid backbone – this is a mechanism for additional selection.

All in all, gel purification steps should be unnecessary.

The BioBrick Prefix and Suffix

Normal BioBrick Prefix: gaattc *gcggccgc* t tctaga g
EcoR1 **Xba1**

Protein Coding Sequence Prefix: gaattc *gcggccgc* t tctag
EcorR1 **Xba1 (incomplete)**

Normal BioBrick Suffix: t actagt a *gcggccg* ctgcag
Spe1 **Pst1**

Notes on Prefix and Suffix Construction

- Every prefix and suffix has a spacer between the two available restriction sites, in order to improve restriction enzyme efficiency.
- The normal prefix has an extra base, (G), at the end in order to avoid creating a methylation sequence when a part is placed next to it; transforms the open GA into a closed GAG.
    - **Methylation sequence:** (GATC), for the *dam1* methylase enzyme.
- The normal suffix has an extra base, (T), at the beginning for the same reason.
    - The methylation sequence in this case is different; targetting the *EcoK/B1* methylase enzyme.
- The modified prefix is missing an (A·G) at the end, immediately next to the part. This setup allows the correct spatial arrangement to result when an RBS is ligated to the beginning of the protein coding sequence (results in a 6 b.p. scar).
    - This setup is possible since protein coding sequences always begins with an ATG. This (A) can then be used to complete the Xba1 restriction site of TCTAGA.

Protein Fusion Issues

When trying to fuse two protein domains, certain issues arise. When reading this section, it is important to note that protein domains, unlike protein coding sequences, do not always begin with ATG (start codon) and do not always end with a stop codon (TAG, TAA, TGA).

- **Frameshift:** since the second protein domain should not contain a redundant start codon, the resulting scar between protein domains is 8 b.p. long (TACTAGAG), which obviously shifts the reading frame.
- **Stop codon:** under the modified frame, nucleotides 4-6 (TAG) form a stop codon.

- Note that this is not an issue under normal constructions – since the frameshift does not occur in RBS-CDS ligations, the stop codon does not appear; also, stop codons should be irrelevant in terms of ligations between promoter sequences, RBS sequences, regulatory elements, etc., since these are untranscribed regions.

"The Silver Standard" is a common solution to this issue, whereby removal of the extra (G) and (T) results in the formation of a 6 b.p. scar, which does not include a stop codon.