

BBF RFC 46: Large-Scale Peptide Modification on BioBrick Proteins

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1. Purpose

With the established BioBrick Assembly standards, fusion proteins with diversified functions are easy to manipulate. However, we realized that the existing BBF RFC do not clearly defining the standard for peptide display on BioBrick proteins. Considering the promising use of specific small peptide modification on functional proteins, targeting to specific cell types, for example, we proposed a standard for small peptide modification on BioBrick proteins.

2. Relation to other BBF RFCs

This BBF RFC extends BBF RFC 10 and BBF RFC 25. For fusion proteins of large segments, BBF RFC 37 is recommended.

3. Copyright Notice

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4. Definition

4.1 Small Peptide Sequences

Small peptide sequences are defined as the peptide sequences that are smaller than 50 bp.

4.2 Definitive Peptides

Definitive peptides are the peptide with known sequences. They are also RECOMMEND to have known functions.

4.3 Randomized Peptides

Randomized peptides are the peptide with randomized sequences, which are often generated for large-scale screening of peptides with desired specificity.

4.4 Interchangeable Peptides

Peptides with standardized prefix and suffix for protein fusion that can be replaced by other BioBricks that also accords this standard.

5. Motivation

As for peptides displayed onto the BioBrick proteins, they should be easier to be changed to meet different specificity. Some peptides, such as those generated by phage display, are relatively hard to be incorporated onto the existing BioBrick protein due to their small encoding sequences. Researchers can modify the desired BioBrick protein via PCR amplification of their original sequence(s).

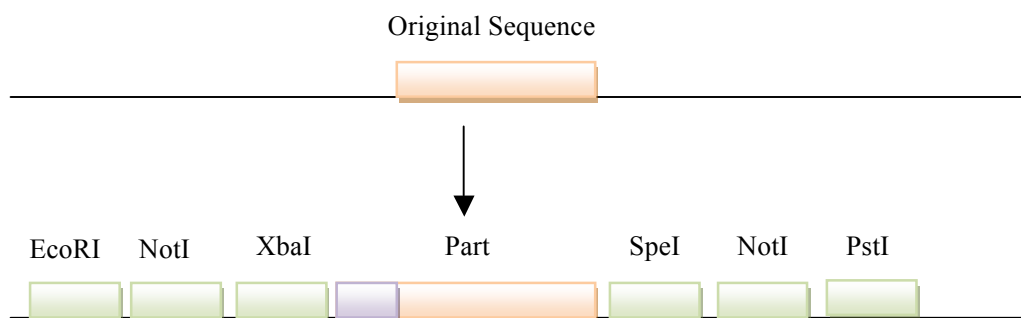


Figure.1 Original Procedure for Small Peptide Modification on BioBrick Proteins

However, when researchers want a certain BioBrick Protein to be modified by a large variety of small peptides, either definitive or randomized, the original procedure will not be easy to manipulate.

Thus, we designed the standards for large-scale peptide sequence modification regarding both definitive and randomized sequences.

6. Definitive Peptide Sequence Modification

When dealing with large-scale definitive peptide modification, researchers are RECOMMENDED to firstly make a “Mother Part” without any peptide modification.

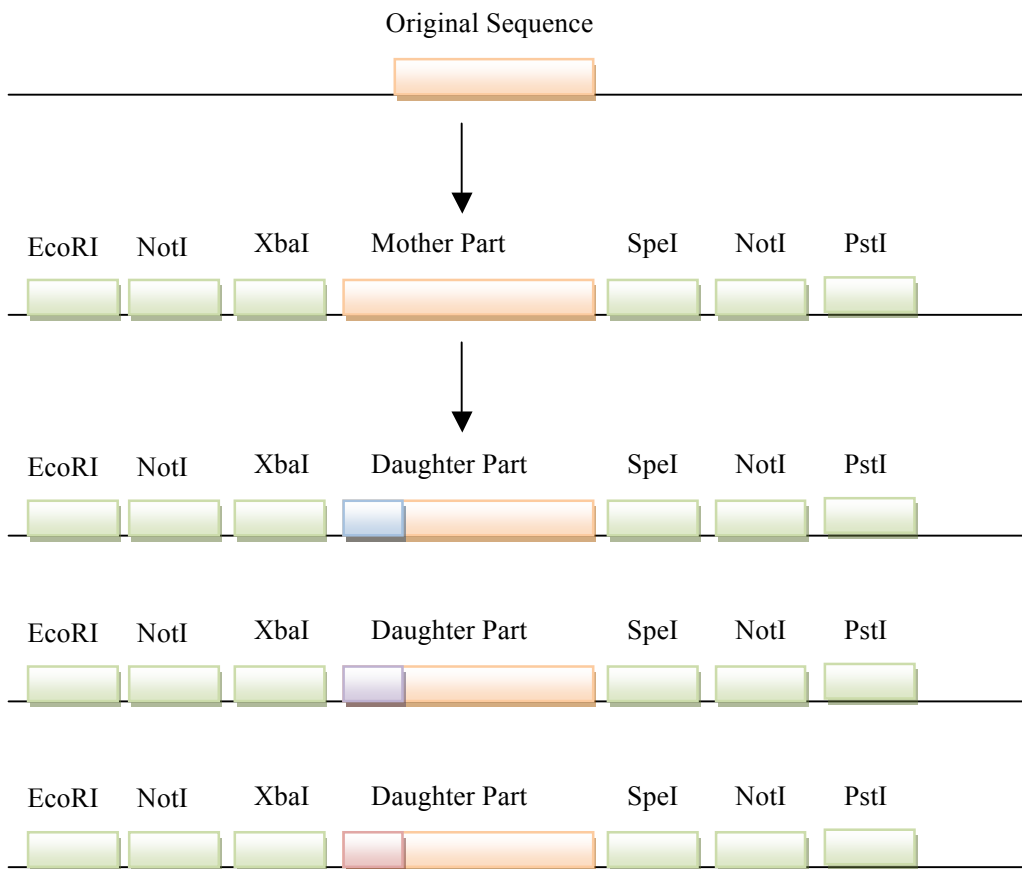


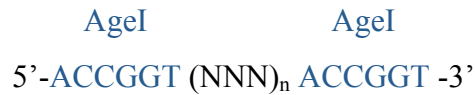
Figure.2 Standardized Procedure for Definitive Small Peptide Modification on BioBrick Proteins

According to BBF RFC 25, Biobricks for protein fusion MUST contain NgoMIV as the prefix and AgeI as the suffix. The construction of Mother Parts MUST also contain NgoMIV as the prefix and AgeI as the suffix if they are supposed for protein fusion.

7. Randomized Peptide Sequence Modification

As for randomized peptides for large-scale specificity screening, the peptide sequences are RECOMMEND to be incorporated into the BioBrick protein as the following standards.

For BioBricks that will not experience further fusion, peptide sequences should be randomly generated as:



When the peptides are supposed to modify the upstream region of the Mother Part,



When the peptides are supposed to modify the downstream region of the Mother Part

Then, the procedure for peptide modification SHOULD be as showed in Figure.3 or Figure.4.

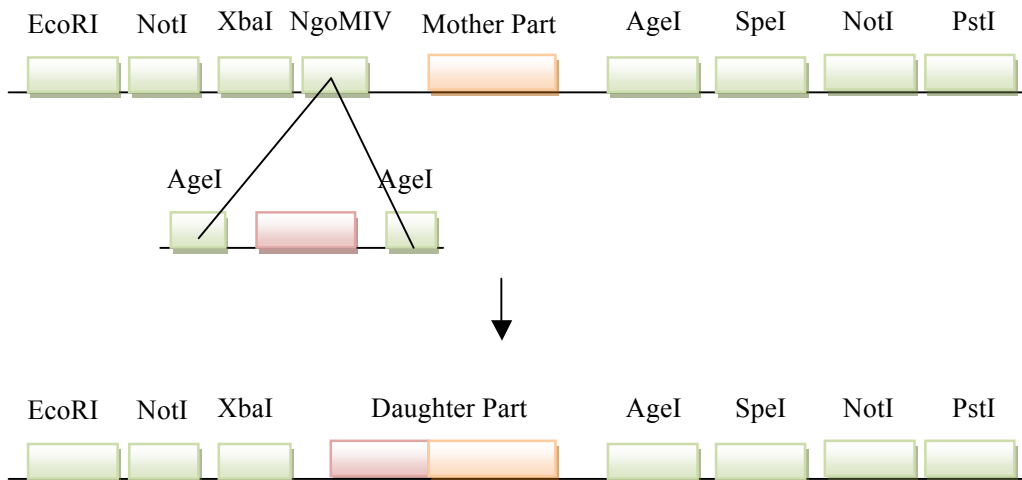


Figure.3 Prefix-Modification of Randomized Peptide Sequence

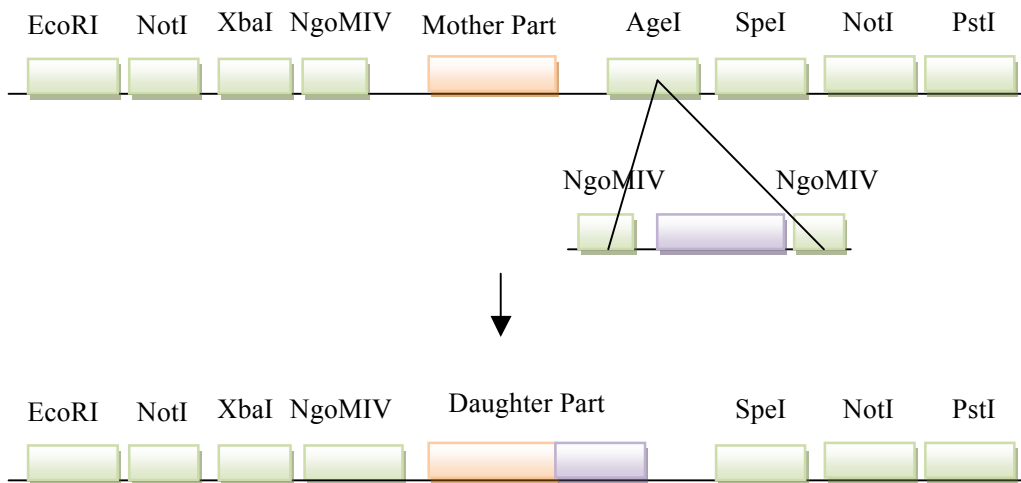
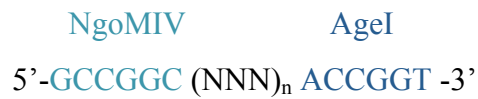


Figure.4 Suffix-Modification of Randomized Peptide Sequence

For BioBricks that need further fusion, peptide sequences should be randomly generated as:



Then, the procedure for peptide modification SHOULD be as showed in Figure.5 or Figure.6.

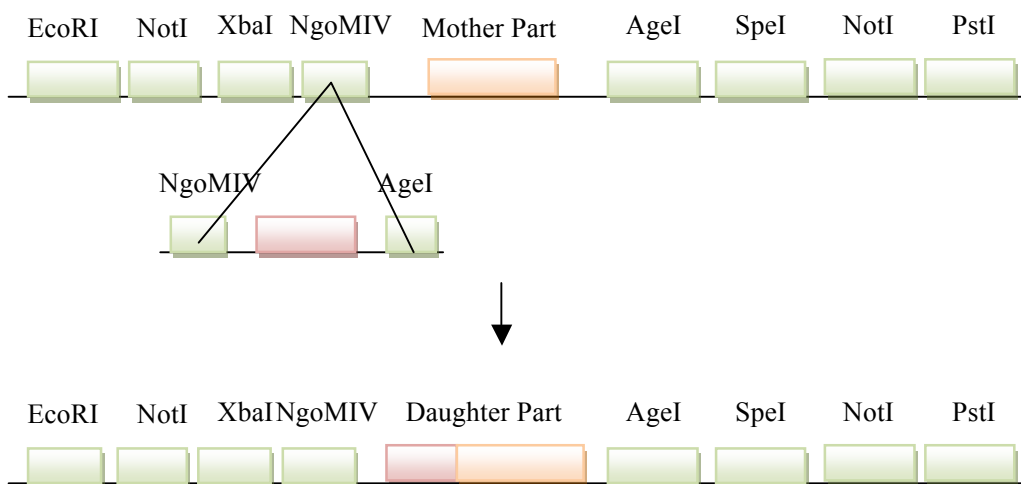


Figure.5 Prefix-Modification of Randomized Peptide Sequence for Further Fusion

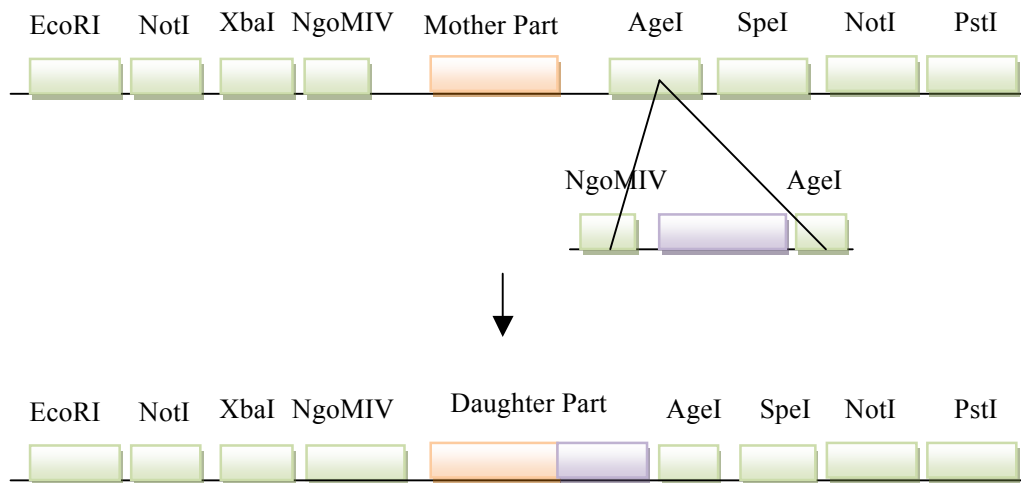


Figure.6 Suffix-Modification of Randomized Peptide Sequence for Further Fusion

8. Summary

- BBF RFC 46 is an extension of BBF RFC 25 and compatible with BBF RFC 10.
- It makes large-scale peptide modification easier to manipulate.
- The fusion format is compatible with the established BioBrick cloning protocols preserving standard restriction sites of prefix and suffix.

9. Author's Contact Information

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References

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