

BBF RFC 87: A method for making constructs with interchangeable regions in the middle

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1. Purpose

This Request for Comments (RFC) describes the method for making BioBrick constructs with cloning sites for BioBricks in the middle using Type IIS restriction enzymes.

2. Relation to other BBF RFCs

BBF RFC 87 does not update or replace any earlier BBF RFC. However the technique is related to RFC28.

3. Copyright Notice

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4. Description

This technique based on Type IIs (T2S) restriction enzymes which can generate four base overhangs of desired sequence. By incorporating two T2S restriction sites (T2S cloning site) in the BioBrick construct (Fig.1) it is possible to clone desired BioBrick (insert). This can be applied for screening of inserts. Insert can be bio fused which makes it useful for protein domain screening.

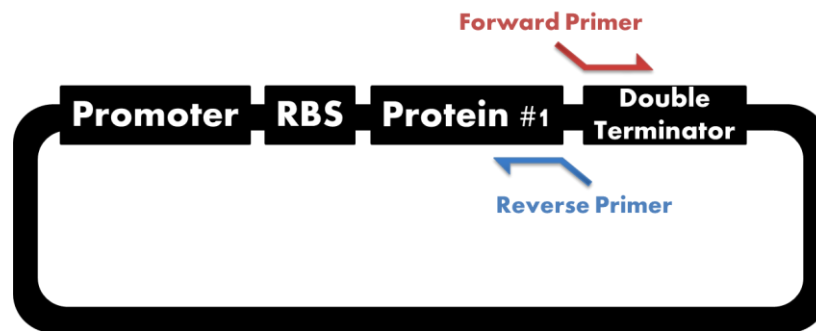


Figure 1 A BioBrick construct with primers to insert T2S cloning site.

5. Methods

Following is an example how to make T2S backbone and clone the desired insert. Bsa I a T2S restriction enzyme will be used for a cloning site. Easiest way to add T2S cloning site is with primers via PCR. T2S cloning site will be inserted between protein #1 and double terminator in (Fig.1). We will call the resulting construct T2S backbone (Fig.2).

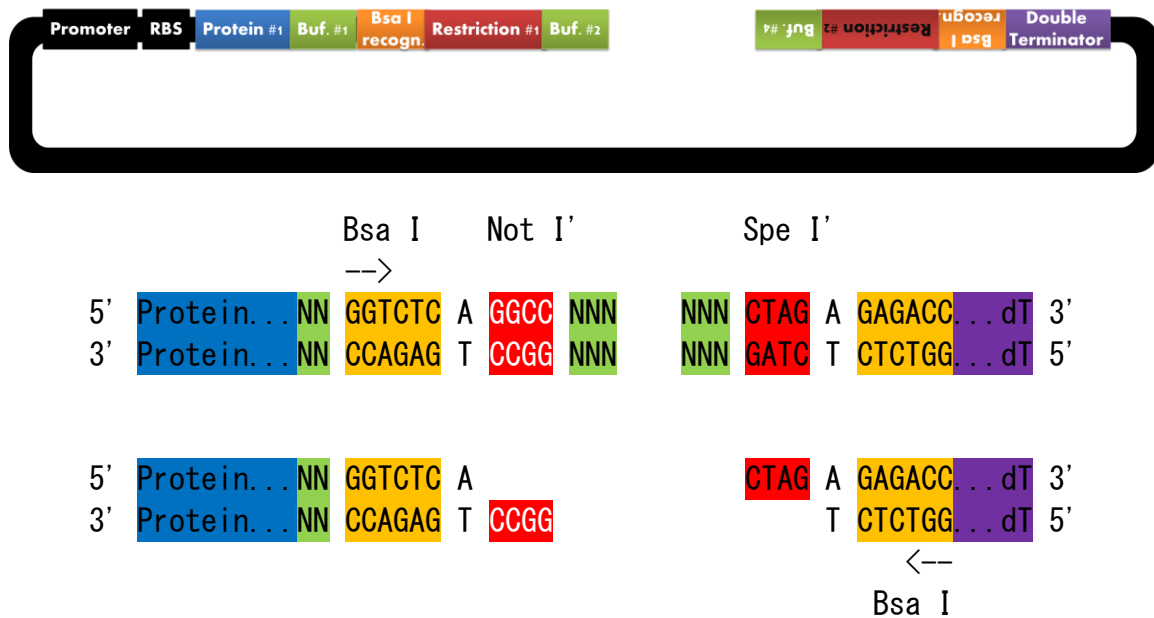


Figure 2. Amplified T2S backbone and sequence of the cloning site.

The primers from figure 1 required to construct this T2S backbone are shown below (Fig.3)

Forward:

5' NNN CTAG A GAGACC... dT 3'

Reverse:

5' NNN GGCC T GAGACC NN...nietorP 3'

Figure 3. Primers to make T2S backbone

Insert BioBrick must be amplified with primers which delete stop codon and Xba I restriction site. The biobrick will be then digested with Not I, Spe I and Dpn I restriction enzymes. Digestion of T2S backbone by Bsa I expose cutting ends designed to anneal to Not I and Spe I. As a result protein encoded in the insert BioBrick would be fused to protein #1 C-terminus.

BioBrick scar between protein #1 and double terminator (Fig. 1) was removed. Forward primer (Fig. 1) is designed to anneal to ending sequence of protein #1. Buf. #1(Fig. 2) is for adjustment of the translational frame of the part which will be cloned into the backbone.

Some enzymes require additional base pairs for efficient restriction. Best practice is to add buffer in these cases, buffer 2 and buffer 4(Fig. 2).

General pointers

T2S cloning site can be inserted anywhere in a construct.

Requirements for insertion of T2S cloning site

- T2S restriction enzyme must be four base cutter.
- There must not be any T2S restriction site already.
 - If there are, delete them or use a different T2S restriction enzyme.
- You must adjust translation frame by adding bases if it is needed.

For make bio fusion of the insert you must consider the following

Fusion to N-terminus: delete Spe I at its stop codon, use Xba I and Not I for insertion.
Fusion to C-terminus: delete Xba I at its stop codon, use NotI and Spe I for insertion.
Fusion at the middle : delete Xba I and Spe I at its stop codon. Using only Not I will yield 50% reverse insertions. If you cannot afford it delete one of the Not I sites and use either EcoR I or Pst I instead of it.

Adding GFP to T2S cloning site and using as a marker for failed ligation should prove convenient.

6. Author's Contact Information

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References

Please see the wiki HokkaidoU_Japan 2011 for the details of practical examples.